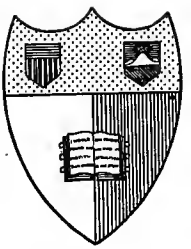
Monographs on Industrial Chemistry

MARGARINE

WILLIAM CLAYTON



MONOGRAPHS ON INDUSTRIAL CHEMISTRY

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INTRODUCTION

DURING the last four or five decades the Applications of Chemistry have experienced an extraordinary development, and there is scarcely an industry that has not benefited, directly or indirectly, from this expansion. Indeed, the Science trenches in greater or less degree upon all departments of human activity. Practically every division of Natural Science has now been linked up with it in the common service of mankind. So ceaseless and rapid is this expansion that the recondite knowledge of one generation becomes a part of the technology of the next. Thus the conceptions of chemical dynamics of one decade become translated into the current practice of its successor; the doctrines concerning chemical structure and constitution of one period form the basis of large-scale synthetical processes of another; an obscure phenomenon like Catalysis is found to be capable of widespread application in manufacturing operations of the most diverse character.

This series of Monographs will afford illustrations of these and similar facts, and incidentally indicate their bearing on the trend of industrial chemistry in the near future. They will serve to show how fundamental and essential is the relation of principle to practice. They will afford examples of the application of recent knowledge to modern manufacturing procedure. As regards their scope, it should be stated the books are not intended to cover the whole ground of the technology of the matters to which they relate. They are not concerned with the technical *minutiæ* of manufacture except in so far as these may be necessary to elucidate some point of principle. In

some cases, where the subjects touch the actual frontiers of progress, knowledge is so very recent and its application so very tentative that both are almost certain to experience profound modification sooner or later. This, of course, is inevitable. But even so such books have more than an ephemeral interest. They are valuable as indicating new and only partially occupied territory; and as illustrating the vast potentiality of fruitful conceptions and the worth of general principles which have shown themselves capable of useful service.

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BY

WILLIAM CLAYTON

M.Sc. (LIVERPOOL)

MEMBER OF THE BRITISH ASSOCIATION COMMITTEE ON "COLLOID CHEMISTRY AND ITS GENERAL AND INDUSTRIAL APPLICATIONS"

WITH ILLUSTRATIONS

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PROFESSOR W. C. Mc. C. LEWIS

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THE AUTHOR

PREFACE

In this monograph, the first of its kind in any language, I have attempted to give a succinct account of the modern processes of manufacture of margarine. The chemistry of its constituents is discussed, and the methods of their analysis, as well as of the finished product, are described in detail. Chapters are devoted to butter and renovated butter, and lard compound. Recent investigations on the so-called Vitamines, especially as these affect butter and its substitutes, have been dealt with in a special chapter on Nutritional Chemistry.

Throughout the text, numerous references have been made to the literature of the subject, and also to the principal English and foreign patents relating to it. A very full bibliography, with due regard to every aspect of margarine technology, has been compiled, which it is hoped will prove of value to research workers in margarine.

My best thanks are due to Prof. W. Ramsden, M.A., M.D., of the Biochemical Department, University of Liverpool, for kindly reading through the proofs of the chapter on Nutritional Chemistry. For the loan of blocks and photographs for illustrating this book, I desire to thank the following firms: N. V. Grasso's Machinefabrieken, 's-Hertogenbosch, Netherlands; Allbright-Nell Company of Chicago; Silkeborg Maskinfabrik (Zeuthen and Larsen) of Denmark.

For kind assistance in facilitating the loan of blocks and photographs, my acknowledgments are due to Mr. Henri Engel (London agent for Grasso), and to Mr. C. Christensen (London agent for Silkeborg).

For the diagrams illustrating the vitamine studies on rats in Chapter XIII, I am indebted to the Publishers and Editor of the *Journal of Physiology*.

In the preparation of the indexes I have been greatly assisted by my wife, to whom my best thanks are due.

W. Clayton.

Liverpool,
July 12, 1920.

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MARGARINE

CHAPTER I

INTRODUCTION

MARGARINE is a fatty food intended as a desirable substitute for butter in the human dietary. It was originally a war-product, created by the Franco-Prussian War, and it may be said to have now established itself as a common article of diet as a consequence of the recent World War. The manufacture of margarine is really a present-day success with a promising future, the former prejudice against the article having been largely dissipated by its more extensive use during the last four or five years.

Shortly before the Franco-Prussian War, a French chemist, Mège-Mouries, was working on the problem of the production of a food which should resemble butter in its chemical and physical characteristics, basing his experiments on the known facts connected with the metabolic changes occurring in the cow, when grass and other foods become changed into milk-fat (butter-fat). Napoleon III had offered a prize for the successful production of a fat which should be as appetizing, nutritious and stable as butter, and caused a factory at Poissy, near Paris, to be placed at the disposal of the inventor of a process. In 1870, Mège-Mouries secured the prize for his invention of oleomargarine. The manufacture soon developed, E. Pellerin buying the sole rights from the French inventor in 1871, and four years later over 300 tons per day were made in Paris alone. In 1873 the Parisian Council of Hygiene sanctioned the sale of such butter-substitutes, at the same time prohibiting the use of the title of "butter" in connection with them. The manufacture

of margarine in Italy was begun in 1874, the foundation of a very large concern being laid in Milan by Regondi and Chierichetti. The first Scandinavian factory was started in 1876 by A. Pellerin.

Since the time of Mège-Mouries many improvements have been made in margarine manufacture, and an extraordinary development has occurred in the machinery for its production. The whole process is now based upon scientific principles and in the light of chemical, physical and biological knowledge.

Mège-Mouries reasoned that in the animal metabolism the carbohydrates contained in the food were converted into fat, the stearine of which was largely diminished during respiration. This fat was then converted into butter-fat by the process of pepsin-digestion. He was thus led to try to effect artificially a similar change in animal fats. Fresh beef fat, usually from the kidney or intestines, free from tissue, was digested for two hours at 45° C., in an aqueous solution of sodium carbonate, in presence of pigs' or sheep's stomachs. As a result of pepsin action, the fat was completely separated from the remaining tissue; it was then skimmed off, and warmed with a 2% solution of common salt to prevent rancidity. On standing, a yellow fat separated, which was cooled to about 22° C. The semi-solid mass possessed a slight butter-odour, and when pressed between warmed plates the more fluid constituent was obtained, averaging about 50–60% of the fat. This fat, when cooled, had a butter-like consistency, and was termed "oleomargarine," derived from the Greek word for pearl (margarites, hard "g"), the cooled product possessing a granular or globular structure.

It was believed that "oleomargarine" was to a great extent composed of "margarin," the triglyceride of margaric acid, which Chevreul regarded as a constituent of certain fats such as human fat and olive oil. Later research showed that the so-called margaric acid ($C_{17}H_{34}O_2$) was not a definite chemical compound, but an eutectic mixture of stearic and palmitic acids. Adopting Chevreul's nomenclature, oleomargarine was considered to be a mixture of olein and margarin, the stearine portion having been removed during the hot pressing.

Mège-Mouries believed he had by artificial means made real butter. But it lacked taste, and he therefore sought to improve its flavour by churning the oleomargarine with 10% of cow's milk, and water containing macerated cow's udder (0·4%). The resulting emulsion was solidified, washed, salted and coloured,

and was sold as a butter-substitute, having a moisture-content of about 12·5%, and a melting-point of 17–20° C.

The first English patent for the manufacture of oleomargarine is that of Hippolyte Mège (2157 of 1869). The abridged specification[1] claims that "a fatty body, identical in chemical composition with butter, is obtained from fresh suet by crushing it between rollers under a stream of water, further washing it, and then digesting it with artificial gastric juice. The fat is extracted, melted, passed through a sieve, and poured into boxes to set, after which it is cut into pieces which are wrapped in cloths and pressed between hot plates. A fatty body is expressed, and may be agitated in a closed vessel, cooled, cut up, bleached with acid, and washed with water. This purified fat is mixed at animal heat with water containing small quantities of bicarbonate of soda, casein of cold milk, and mammary tissues, along with yellow colouring matter. This is digested, allowed to settle, decanted, and cooled, and yields a preserved butter. Fresh butter is obtained by agitating the above mixture until a cream is formed, which is then treated as usual to obtain the butter."

An interesting account of the early manufacture of oleomargarine or "butterine," as it was also called, in the United States of America, was given in *Nature* in 1882.[2] The yield of oleomargarine was found to be about 35% of the beef caul fat employed, and the product is described as having a taste similar to that of second-grade butter, but being rather more salt. Annatto was used as the colouring matter, and the butterine emulsion was rolled in ice to set it, before being packed in kegs. Even as early as 1879 over 6000 tons of oleomargarine were exported to Europe from New York, of which 5300 tons were cleared for Rotterdam and 480 tons for Liverpool.

Since 1871 the manufacture of margarine has steadily progressed, numerous oils have been introduced, and modern methods and machinery employed. The fat is no longer digested artificially, the butter flavour being introduced by suitable treatment of the milk used in churning with the fatty constituents. Instead of a single fat, "oleomargarine," other animal fats, such as stearine, "premier-jus," and lard, have been used, and about fourteen years ago margarines made from purely vegetable oils and fats were placed on the market. The first mention of mixing

[1] Abridged Patent Specifications, Class 84, 2157 (1869).
[2] *Nature*, **25**, 269–70 (1882).

beef-fat with some suitable oil, such as cottonseed oil, was made by Rondebush in 1873.[1] Hofmann,[2] in 1880, suggested the use of ground-nut oil, sesamé oil, and hazel-nut oil. Margarine made from vegetable fats was patented in 1883 by Meinert and Jeserich,[3] and in 1896 the employment of coconut oil, now so extensively used in margarines, was claimed by Ruffin.[4]

Variations of the original patent by H. Mège were early taken out by Diderischen,[5] Hipkins,[6] Dordron,[7] Brewer,[8] and Cochran.[9]

From the purely scientific side, four great advances are noteworthy in margarine technology. They are: (1) the use of commercial cultures of lactic acid organisms for souring the milk used in churning, and thus imparting a butter flavour to the finished product; such cultures were independently suggested by Storch and Weigmann in 1890; (2) the introduction of vegetable oils and fats leading to the so-called "nuts and milk margarines," being placed on the market about 1906. Warr and Wright in 1900[10] described a typical "nuts and milk margarine"; (3) the introduction in recent years of hydrogenated oils, whereby a new and extensive source of raw (fatty) material is opened up to margarine manufacturers;[11] (4) the use of artificial milk, made from vegetable oils and nuts is a distinct advance, and margarine can now be made, in which a purely artificial milk is pasteurized, soured and emulsified, yielding a product quite equal to that made with the usual separated cow's milk.[12]

From the practical standpoint, the most striking improvements have been: (1) the use of a sheet,[13] and later, of a spray,[14] of ice-cold water, to solidify the margarine emulsion; (2) the employment of a brine-cooled rolling drum, notably that of Schou,[15] whereby the emulsion is rapidly cooled out of contact with the cooling medium; (3) the introduction of a continuous emulsifier or churning apparatus, effecting much economy in time and space. A notable example is the electric continuous churn of the Silkeborg Maskinfabrik Zeuthen and Larsen[16]; (4) the use of butter-working tables, mills, drums, blenders, and other devices for rolling or kneading the margarine.

Finally, the whole question of margarine as a substitute for

English Patents:—

[1] 4209 (1873). [2] 3867 (1880). [3] 915 (1883).
[4] 1827 (1896). [5] 661 (1874). [6] 664 (1874).
[7] 1049 (1874). [8] 1547 (1881). [9] 3892 (1882).
[10] 22,602 (1900). [11] 18,376 (1913). [12] 24,050 (1911).
[13] 20,438 (1896). [14] 22,873 (1896). [15] 12,561 (1907).
[16] 4657 (1914).

butter is being reviewed in the light of knowledge gained within the last ten years in the field of bio- and nutritional-chemistry. The discovery of " vitamines " or " accessory-food substances " has introduced a new factor, and it has been suggested that definite standards or limits should be placed on the nature and composition of margarines in general, which should have regard to their vitamine content and consequent growth-promoting qualities.

CHAPTER II

OILS AND FATS USED IN MARGARINE MANUFACTURE

Arachis Oil.—This oil is obtained from the ground-nut (*Arachis hypogæa*, Linn.), also known as the earth-, monkey-, and pea-nut. It is extensively cultivated in tropical and sub-tropical countries, especially in the Far East, West Africa, India and North and South America. In 1917 the year's supply of ground-nuts to all European mills exceeded 600,000 tons.

The average oil-content of the ground-nut is about 48%, but varies with the conditions of plant growth, *e. g.* the richness of the soil. The best edible oil is cold-drawn (yield about 18%), and this may be bleached to nearly water-white by means of decolorizing charcoal or fuller's earth. English refined oil is usually a pale yellow colour. The oil deposits a "stearin" on standing in cold weather. For use in margarines the best oil only is permitted, possessing a slight but pleasant nutty odour and taste, and a free, fatty acid content of 0·1–0·3% as oleic acid. The best qualities of ground-nut oil are the Rufisque (Senegal) and Gambia. Prior to the war, Marseilles, Bordeaux and Delft were noted manufacturing centres for high-grade arachis oils.

The specification for the best edible arachis oil in the United States of America as defined by the Interstate Crushers' Association is as follows: "Choice pea-nut oil must be sweet in odour and flavour, prime in colour, clear and brilliant in appearance and free from moisture, and shall not contain more than one-tenth per cent. of free fatty acid. Prime yellow pea-nut oil must be clear, sweet in odour and flavour, free from water and settlings, and of no deeper colour than fifty yellow and five red on Lovibond's equivalent colour scale."

Adulterants sometimes present in arachis oil are cottonseed oil, poppyseed oil, rape oil, maize oil and sesamé oil. Cotton-seed oil is detected by the Halphen reaction. Poppyseed oil will raise the iodine value and the specific gravity. Rape oil

ANALYTICAL CONSTANTS OF OILS AND FATS USED IN MARGARINE

Oil or Fat.	Melting Point.	Solidifying Point.	Titer.	R.X. at 40° C.	I. Val.	Sap. Val.	S.G. $\frac{15}{15}$.	S.G. $\frac{99}{15}$.	R.M.	Pol.	K.	Unsap.
Arachis Oil	—	—	27–30° C.	55–57·5	87–98	190–196	0·916–0·918	—	0·5	—	—	0·4–0·8 %
Babassu Oil	26° C.	—	23° C.	—	15·6	249	—	0·868	5·8	10·2	—	0·3
Butterfat	28–34° C.	19–24° C.	33–37° C.	38–42	26–38	220–232	0·936–0·942	—	26–33	1·7–3·0	20–26	0·3–0·45
Cocoa butter	32–34° C.	27–29·5° C.	48–50° C.	46–47·5	35–40	190–198	0·968	—	—	—	—	0·8–1·2
Coconut Oil	23–26° C.	22–23·5° C.	21–26° C.	34·7–36·0	8–10	255–260	—	0·869–0·874	6–8	15–18	1·6–1·9	0·15–0·3
Coconut Stearine	23–32° C.	22–29° C.	—	34·6–36·0	2–10	250–260	—	0·860–0·874	—	—	—	—
Cohune Oil	22–24° C.	19–21·5° C.	25–29° C.	35·2–36·2	9–12·5	254–260	—	—	—	—	—	—
Cottonseed Oil	—	—	35–38° C.	58–59	105–115	192–195	0·922–0·926	—	0·2–1·0	0·4	—	0·7–1·7
Cottonseed Stearine	—	19° C	—	—	91·2	196·2	0·919	—	—	—	—	—
Kapok Oil	—	—	29–36° C.	53–57	95–110	190–197	0·921–0·923	—	—	—	—	—
Latifolia Fat	—	—	—	47·7	59·4	192·2	—	0·8595	—	—	—	—
Lard	36–48° C.	27–30° C.	36–42° C.	49–52	47–85	195–203	0·933–0·938	0·860–0·862	—	—	—	0·2–0·4
Lard Oil	—	−4° to +10° C.	—	41·0	67–88	190–198	0·913–0·918	—	—	—	—	0·3–0·5
Linseed Oil	−16° to −20° C.	−27° C.	19–20·5° C.	72–75	175–200	189–195	0·932–0·938	—	—	0·1	—	0·8–2·0
Longifolia Oil	—	—	—	49·3	62·6	190	—	0·8624	—	—	—	—
Maize Oil	—	−10° to −36° C.	18–20° C.	59·5–60·5	115–125	189–193	0·921–0·928	—	0·29	0·38	—	0·8–3·0
Niger Seed Oil	—	−9° C.	26–28° C.	63–64·5	128–134	189–192	0·925–0·927	—	—	—	—	—
Oleo Oil	30° C., variable	indefinite	—	47·5–48·7	40–50	198–202	—	—	—	—	—	—
Oleo Stearine	50–54° C.	47–50° C.	48–51° C.	46·5–47·5	18–25	192–197	—	—	—	—	—	—
Palm Oil	25–50° C.	31–39° C.	41–49° C.	41–45·0	52–58	196–205	0·920–0·924	0·856	0·5	0·4	—	—
Palm Kernel Oil	25–29° C.	24–26·5° C.	25–28·5° C.	36–37·5	14–24	242–254	—	0·859–0·871	5–7	9·5–10·5	1·0	0·22
Premier Jus	47–49° C.	32–35° C.	42·5–44° C.	47–48	38–45	195–200	—	—	—	—	—	—
Paraguay Nut Oil	—	—	21° C.	—	28·5	247	—	0·865	6·5	10·2	—	0·3
Rape Oil	—	−10° to −16° C.	12–20° C.	59–60	97–106	170–179	0·913–0·917	—	—	—	—	0·5–1·0
Sesamé Oil	—	−4° to −6° C.	22–24° C.	59·5–60	103–115	188–193	0·923–0·926	—	—	—	—	0·8–1·2
Safflower Oil	—	−18° C.	16–17° C.	63–65	135–145	190–194	0·926	—	—	—	—	0·7
Shea Nut Oil	37–42° C.	25–30° C.	54° C.	55·5°–56·5	54–63	179–184	—	—	—	—	—	4–10
Shea Nut Oleine	—	24° C.	—	58·7	62·3	181·6	—	—	2·60	0·72	—	4–10
Shea Nut Stearine	55·5° C.	34·2° C.	—	52·7	51·9	179·7	—	—	—	—	—	4–10
Soya Bean Oil	—	−7° to −12° C.	26–29° C.	62·5–63·5	126–135	190–192·5	0·924–0·926	—	—	—	—	0·5
Sunflower Oil	—	−16° C.	22–24° C.	60–63	129–133	189–193	0·924–0·926	—	—	—	—	—
Teaseed Oil	—	−5° to −12° C.	—	—	89–94	188–196	0·917–0·927	—	—	—	—	—
Tucan Nut Oil	30·5° C.	—	27° C.	—	11·6	249	—	0·867	3·8	5·9	—	0·3

lowers the saponification value and the titer result. Maize oil raises the iodine value and lowers the titer result, whilst sesamé oil is detected by the Baudouin test.

Arachis oil contains olein, linolin, stearin, arachidin and lignocerin. The presence of palmitin is uncertain. The chief acid is arachidic (5%), whilst it is still an open question whether hypogœic acid [1] is present.

The test for arachis oil is based on the quantitative estimation of the mixed arachidic and lignoceric acids. The test was devised by Renard,[2] and has been modified by Archbutt,[3] Bellier [4] and Evers.[5] The test becomes unreliable in the presence of solid animal or vegetable fats (excluding coconut and palm kernel oils), and consequently must be employed with reserve in margarine analysis.

Babassu Kernel Oil.—This is a new fat obtained from the kernel (*Coco babassu* and *bassoba*) of a species of *Attalea*, probably *Attalea funifera*, Mart, which grows plentifully in Brazil. In 1916 nearly 1500 tons of the kernels were exported. They contain about 67% of creamy fat resembling coconut and palm kernel oils, melting at 26° C. The analytical constants are remarkably like those of coconut oil. It is anticipated that this fat will figure prominently amongst vegetable fats intended for margarine manufacture in the near future.

Coconut Oil.—Coconut oil is a white fat obtained from the pulp of the ordinary coconut, the seed of *Cocos nucifera* and *Cocos butyracea*, grown extensively in Ceylon, India, Philippines, South America, South Sea Islands and Cochin China. There are numerous trade names employed for this substance, *e. g.* "nucoline," "loderline," "velcolene," "nutto," "vegetaline," "cocolardo," "palmine," "neutrex," "lactine," "laureol," also "vegetable lard," and "nut lard."

Coconut oil intended for margarine production is a semi-solid, white mass of very low free-fatty acid content, on an average about 0·07% as lauric acid. The fat is practically devoid of smell and taste. It is not often adulterated, though palm kernel oil may be added, or the "coconut stearin" partially removed. The fat is commercially graded as Cochin, Ceylon and Copra oil, the best grade oils being all classified as Cochin oils. The specification of the Interstate Crushers' Association

[1] Meyer and Beer, *Monatsch. f. Chemie* (1913), 1195.
[2] *Comptes rendus*, **73**, 1330 (1871).
[3] *J.S.C.I.*, **17**, 1124 (1898)
[4] *Ann. Chim. Anal.*, **4**, 4 (1899).
[5] *Analyst*, **37**, 487 (1912).

(U.S.A.) concerning "choice grade" or "Cochin grade" oils, states that they must be "free from moisture and impurities, and shall have a colour not greater than six yellow and one-half red."

Coconut oil is characterized by a high Reichert-Meissl value (about 8) and Polenské value (about 16), and also by a high saponification value (about 257). It also possesses a low iodine value, and is readily soluble in alcohol (Hink's test).[1]

Elsdon,[2] by an alcoholysis method of analysis, arrived at the following approximate composition of the mixed fatty acids of coconut oil: caproic acid = 2%; caprylic acid = 9%; capric acid = 10%; lauric acid = 45%; myristic acid = 20%; stearic acid = 5%; palmitic acid = 7%; oleic acid = 2%.

Coconut stearine is the product obtained by melting coconut oil, and then slowly cooling it so as to induce crystals or "grains" to disperse throughout the mass. Subsequent pressure in a hydraulic press causes the liquid "oleine" to run out, and the more solid "stearine" is retained. This product is sometimes employed in margarines, but more often in cooking fats, such as lard compounds.

Cohune Oil.—Cohune oil is obtained from the kernels of the cohune palm, *Attalea cohune*, Mart, extensively grown in Honduras, Brazil, Guatemala, Guiana and Mexico. A synonymous name is the Manacca nut. The seed contains 65–72% of a white solid fat, melting at about 23° C., of pleasant nutty flavour. Its analytical constants closely resemble those of coconut oil, and doubtless in the near future cohune oil will play a not unimportant rôle in the choice of vegetable fats for margarine manufacture. In this connection it is interesting to note that it has been estimated that a single area of the cohune palms, extending along the Hondurasian River Anguan for sixty to seventy miles and ten to twelve miles wide, would easily supply 10,000 tons of nuts per month.

A very similar product is known as coquito or coquilla oil, also obtained from a species of *Attalea*.

Cottonseed Oil.—This oil constitutes about 20% of the seeds of the cotton plants which are species of *Gossypium*, e. g. *Gossypium hirsutum* (U.S.A.), and *G. barbadense* (Egypt). The chief productive areas are the United States of America, Egypt, India, Brazil, Peru, Russia and West Africa. The finest edible oil is bleached with fuller's earth, yielding a very pale straw-tinted

[1] *Analyst*, **32**, 160 (1907). [2] *Ibid.*, **38**, 8 (1912).

oil of slight but distinctive taste; it is practically odourless. A deposit of "stearin" ensues on standing, and if this is removed a "winter" oil is obtained, *i. e.* it remains quite clear and deposits no further "stearin" on standing in the cold. Such winter oils are also said to be "demargarinated," and they will always give a lower titer result. The highest quality cottonseed oil is known in trade as "butter oil," and plays a most important part in the composition of margarines and lard substitutes.

The American standard for edible cottonseed oil as defined by the Interstate Crushers' Association demands that: "Choice summer yellow cottonseed oil must be sweet in flavour and odour, prime in colour, clear and brilliant in appearance and free from moisture. Prime summer yellow cottonseed oil must be clear, sweet in flavour and odour, free from water and settlings, and of no deeper colour than 35 yellow and 7·1 red on Lovibond's equivalent colour scale. Prime winter yellow cottonseed oil must be brilliant, free from water and settlings, sweet in flavour and odour, and must stand limpid at a temperature of 32° F. for five hours." It must also be of no deeper colour than that of prime summer yellow oil as described above.

Cottonseed oil has no definite chemical composition. The solid acids constitute about 20–25% and are mainly palmitic acid, with possibly some arachidic acid.[1] The liquid acids contain linolic and oleic acids in amounts about 17–18%.

Tests: (1) The principal routine test employed is due to Halphen.[2] The oil is heated in a rapidly boiling water-bath with an equal volume of a 2% solution of flowers of sulphur in carbon disulphide, containing a drop of pyridine.[3] A closed tube increases the delicacy of the test, which shows a crimson colour, the intensity of which depends on the quantity, variety and previous treatment of the oil. The test fails with oils heated to high temperatures, and also with hydrogenated oils.

(2) The Becchi test,[4] modified by Milliau,[5] serves to distinguish cottonseed oil from kapok oil, which is a very similar oil also giving the Halphen reaction. The fatty acids from 15 c.c. of the oil (obtained by saponifying with NaOH and alcohol, dissolving in 200 c.c. of water, boiling off the alcohol, and adding $\frac{N}{10}$ H_2SO_4 in slight excess) are carefully washed with 15 c.c. of

[1] Meyer, *Chem. Zeit.* (1907), 794.
[2] *J. Pharm. Chim.*, **6**, 390 (1897).
[3] Gastaldi, *Chem. Zeit.*, **2**, 758 (1912).
[4] *Zeit. Anal. Chem.*, **33**, 560 (1894).
[5] *J.S.C.I.*, **12**, 716 (1893).

cold distilled water twice and then quickly dried at 105° C. To 5 c.c. of these fatty acids is added an equal volume of a 1% solution of silver nitrate in absolute alcohol. On shaking the mixture a very slight brown colour may be given by cottonseed oil, whilst a deep coffee-brown colour will rapidly develop if kapok oil be present.

(3) Cottonseed oil, shaken with an equal volume of strong nitric acid and allowed to stand for twenty-four hours, develops a brown coloration.

If cottonseed oil is chilled, a deposit is rapidly formed, consisting of "cottonseed stearine," which is chiefly palmitin, stearin (about 4%), and a varying proportion of liquid glycerides. Small quantities of arachidic acid are also present in this deposit. Commercially, "cottonseed stearine" is known as "cottolene" or "vegaline," and is used in making lard compounds, and certain margarines for confectionery purposes.

Kapok Oil.—This oil, which, as already stated, is very closely related to cottonseed oil, is a product of the seeds of *Eriodendron anfractuosum* and *Bombax malabaricum*, indigenous to Java, Africa and the West Indies. The *E. anfractuosum* is locally known as the "silk-cotton tree." The expression of kapok oil for margarines has so far been a Dutch industry, but the oil is rapidly coming into more extended use, especially in America. Kapok oil is usually darker in colour than cottonseed oil, but otherwise resembles it in taste and odour to a remarkable extent. It responds to the Halphen test, but may be differentiated by means of the Becchi test as modified by Milliau (*vide* cottonseed oil).

Lard.—Of the several grades of lard obtained by the American continuous method of extracting the fat of pigs, only the first two, viz. neutral lard No. 1 and neutral lard No. 2, are employed in margarine manufacture. The No. 1 lard is prepared from the leaf, and No. 2 from the back fat, and in each case is rendered between 40–50° C. The free fatty acids should not exceed 0·25% (as oleic acid). The use of lard in margarines gives a high-quality product, and it is especially employed in oleomargarines.

Lard is a white, translucent, pasty mass, the best qualities possessing a characteristic granular structure. These grades, too, are practically free from taste and odour.

The chemical composition of lard varies with the breed of the pig, its food, and the part of the body whence it was extracted.

The fatty acids contain about 40% solid and 60% liquid acids. The solid acids include myristic, lauric, stearic and palmitic, whilst oleic and linolic acids (ratio 5 : 1) constitute the liquid acids. According to Farnsteiner[1] traces of linolenic acid are also present.

Systematic adulteration of lard has been practised, and, in consequence, lard requires to be examined for moisture, cotton-seed, maize and sesamé oils, coconut and palm kernel oils, and beef and mutton fats. The following tests may be applied—

(*a*) *Moisture.*—Ten grams of the sample are heated at 110° C. to constant weight, and the loss determined. The loss due to moisture seldom exceeds 0·3%. A rapid method, based on the temperature at which the melted lard becomes turbid, was devised by Polenské[2] and its validity confirmed by Fischer and Schellens,[3] who give the following results—

Water % . . .	0·45	0·40	0·35	0·30	0·25	0·20	0·15
Turbidity temperature °C	95·2	90·8	85·0	75·8	64·6	53·2	41·2

(*b*) *Vegetable oils* in small amount may be detected by the phytosteryl-acetate test, and cottonseed and sesamé oils recognized by their colour tests. Hogs fed on cottonseed- or sesamé-cakes will yield lard responding to the colour tests of Halphen and Baudouin respectively. If the oils are really present, the iodine value should be raised. Cotton oil being absent, maize oil may be detected by the solidification point of the fatty acids, which will be lowered; at the same time the iodine value of the liquid fatty acids will be raised. Coconut and palm kernel oils may be detected by the Reichert-Meissl and Polenské values, and by the increase in the saponification value.

(*c*) *Animal Fats.*—Unless the amount of adulteration exceeds 10% it becomes a difficult matter to express a definite opinion on the nature and extent of admixture with animal tallows. The main test consists in a microscopical examination of the crystals obtained from an ethereal solution of the lard. Such lard-crystals show characteristically chisel-shaped ends, beef and mutton fats under similar conditions yielding fan-like tufts of crystals with pointed ends. The test was devised by Belfield,[4] and later Stock[5] attempted a quantitative modification. It has been shown by Dunlop[6] that repeated crystallizations of

[1] *Zeitsch. Nahr. Genussm.*, **2**, 1 (1899).
[2] *Arb. a. d. Kaiserl. Gesundh.*, **25**, 505 (1907).
[3] *Zeitsch. Nahr. Genussm.*, **16**, 161 (1908).
[4] *Analyst*, **13**, 70 (1888).
[5] *Ibid.*, **19**, 2 (1894).
[6] *J.S.C.I.*, **25**, 459 (1906).

crystals of beef and mutton fats will eventually convert the crystals into the flat and chisel-shape form of lard crystals. Hence caution is required in employing this test.

Lard Oil.—Lard oil is a practically water-white product of very faint odour, obtained by submitting the softer varieties of lard to hydraulic pressure; the residue constitutes "lard stearine" or "pressed lard." Lard oil contains olein, stearin and palmitin, together with linolin. The fatty acids contain from 19–25% solid acids. Lard oil is used in margarines, but more extensively in the manufacture of lard substitutes. The free fatty acid figure will usually be below 0·7% calculated as oleic acid.

Linseed Oil.—The seeds of the flax plant, *Linum usitatissimum*, growing in Central Asia, Argentine, India, North America and Russia, contain from 36–42% of linseed oil. By cold expression a clear, golden-yellow oil of characteristic but not unpleasant smell is produced, which is suitable for edible purposes. The oil deposits no "stearine" on standing, even at temperatures as low as −12° C. The determination of the iodine value affords an important indication of the purity of linseed oil; it ranges from 170–200. A characteristic property of the oil is its ready drying on exposure to the air, whereby "linoxyn," a tough elastic solid, is formed. The chemical constitution of the oil is still somewhat doubtful, but from a critical summary of the published data, Friend [1] gives the following as the composition of linseed oil of iodine values ranging from 170–180 :—

Constituents.	Per cent.	I. Value.	Per cent.	I. Value.
Unsaponifiable matter . .	} 10	0	10	0
Saturated organic acids . .				
Oleic acid	5	4·5	5	4·5
Linolic acid	59·1	107·2	48·3	87·9
Linolenic acid . . .	21·3	58·3	32·1	87·6
Glyceryl radical . . .	4·6	0	4·6	0
	100·0	170·0	100·0	180·0

Linseed oils, raw and hydrogenated, have been used in margarine manufacture, but not to any noteworthy extent.

Maize Oil.—Maize oil or "corn oil," is obtained from the germs of Indian corn, *Zea mais*, these germs being a by-product of the

[1] *Chemistry of Linseed Oil* (London, 1917), p. 64.

maize-starch industry. The dry germs contain about 53% of oil, which, as used in American margarine factories for colouring the margarines, is a clear, golden-yellow colour, with a persistent nutty odour. The refined oil keeps quite well; a slight "stearine" deposit ensues on standing in cold weather. Analytically, the oil is characterized by a high iodine value and refractive index, and a low titer value (18·5° C.). Also it is usual to find the unsaponifiable matter to exceed 1·0%, due to the presence of lecithin. The fatty acids of maize oil consist of palmitic and arachidic acids (about 7%) and linolic and oleic acids.

Niger Seed Oil.—This oil is obtained from the achenes of *Guizotia abyssinica* (L.), grown in East Africa and in the East and West Indies. The seeds contain from 40–45% of oil, which is golden yellow, and possesses a pleasant nutty flavour; the odour is very slight. The cold-pressed oil only is used for edible purposes.

Oleo Oil and Oleostearine.—The fat from the heart, caul and kidneys of oxen and cows, when rendered at about 40° C., gives a clear product known as "premier jus" which can be fractionated into a more liquid constituent, "oleo oil," and a more solid residue, "oleostearine." Separation of these constituents is usually carried out by hydraulic pressure, after the premier jus has been clarified by treatment with brine and allowed to "grain" or "crystallize" on cooling undisturbed.

Oleo oil has a coarsely granular structure, and a pale yellow colour. The free fatty acid content is usually from 0·3–0·5% as oleic acid, and the melting-point 27–31° C., though this, of course, depends on the pressure and temperature of preparation. Oleo oil is a favourite ingredient in best-grade margarines, and is particularly preferred to-day because of its superiority over vegetable fats in possessing the vitamine known as "fat-soluble A," so essential to nutrition (*vide* p. 136).

"Oleostearine" or "beef-stearine" is a pale yellow or white-coloured hard fat, melting about 50° C. Only a small proportion is utilized in ordinary margarines because of its high melting-point, but it is extensively employed in pastry margarines (melting-point 34–40° C.) and in the manufacture of "lard compound" (*vide* p. 130).

Beef fats contain mixed glycerides, amongst which the following have been separated: oleodipalmitin, stearodipalmitin, oleopalmitostearin, and palmitodistearin. From ethereal solutions, beef fat deposits crystals showing a very characteristic form

under the microscope, viz. fan-like tufts, with needle-pointed ends (cf. Lard, p. 12).

Palm Oil.—This is a product of the oil palm, *Elæis guineensis*, indigenous to West Africa. Other species are also coming into use, *e. g. E. melanocca*, of South America. The pulp contains from 55–65% of oil, which, until recently, was most crudely prepared by natives, so that commercial palm oil was characterized by a very high acid value. More attention is being paid to its extraction under expert direction, and a sound, edible palm oil more suitable for margarine manufacture is now finding its way into commerce.

The best quality, known as Lagos oil, has an orange-reddish colour, and a butter-consistency. It is employed in the American margarine industry as a colouring material, together with corn oil and mustard oil (*vide* p. 92), and consists of 98% palmitic acid and the solid acids of palm oil; oleic, stearic and linolic acids have also been identified. [1]

Palm Kernel Oil.—Palm kernel oil is expressed from the seeds of *Elæis guineensis*, which are obtained after removing the fleshy portion of the palm fruit, which yields palm oil. The seeds or kernels contain from 44–49% of palm kernel oil, a white or very pale yellowish fat, closely akin to coconut oil, but entirely distinct in composition and properties from palm oil. The refined oil is extensively used in margarine manufacture, though coconut oil is usually preferred if obtainable. Both expressed and extracted oils are used, but so far the expressed oil is distinctly better for margarines. In chemical composition, palm kernel oil is very similar to coconut oil, and Elsdon [2] has pointed out that "it might not be possible to distinguish between certain samples of coconut oil and palm kernel oil, even in pure conditions." The following comparative analyses are due to Elsdon :—

	Caproic acid.	Caprylic acid.	Capric acid.	Lauric acid.	Myristic acid.	Palmitic acid.	Stearic acid.	Oleic acid.
Palm kernel oil	2	5	6	55	12	9	7	4
Coconut oil .	2	9	10	45	20	7	5	2

An interesting paper in this connection is also due to Heidschka and Burger.[3] Palm kernel oil possesses a fairly high Reichert-

[1] Nördlinger, *Zeit. f. angew. Chem.*, **5**, 110 (1892).
[2] *Analyst*, **39**, 78 (1914).
[3] *Zeitsch. offentl. Chem.*, **20**, 361 (1914).

Meissl value, viz. 5–7, and a high Polenské value, 9·5–10·5. Its presence in a margarine containing coconut oil is difficult of detection (cf. p. 111).

Premier Jus.—High-grade premier jus is a frequent constituent of oleomargarine. It is the unfractionated fat obtained when the fat of the heart, caul and kidneys of cattle is rendered at a temperature as low as 40° C. (see under "Oleo oil," p. 14). The premier jus, as used in margarine, is a yellow fat, melting at about 47° C. and possessing less than 0·5% of free fatty acids. In oleomargarines only a limited quantity is used, owing to its high melting-point. Great care has to be taken in its selection, so as to avoid a "beefy" flavour in the margarine. Premier jus is a favourite ingredient in fish-friers' cooking-compounds.

Rape Oil.—Rape oil is obtained from the very small seeds of *Brassica campestris*, various species being cultivated in Europe, India and Japan. For edible purposes the oil is obtained by expression in the cold, *i. e.* "cold drawn," and is then a yellow oil, noticeably more viscous than the usual edible oils, and possessing a distinctive flavour, which is not unpleasant. A great future undoubtedly awaits this oil in margarine manufacture.

The oil is characterized by a high viscosity, very low saponification value (170–179), and it may be distinguished from other non-drying, and the majority of the semi-drying oils by the insoluble hexabromide test. A noteworthy feature is its insolubility in boiling acetic acid. Adulteration with closely allied oils, such as Ravison and Jamba oils, is difficult to detect, but linseed and cottonseed oils are more easily recognized. Mineral and rosin oils would be indicated by the estimation of the unsaponifiable matter present.

Rape oil fatty acids contain from 0·5–1·6% of a saturated acid, probably arachidic acid. Rapic, erucic and possibly linolenic acids also occur.

Sesamé Oil.—Sesamé oil or Gingelly oil is extensively used in margarine manufacture, being compulsory up to 10% in several European countries. It is obtained from the seeds of *Sesamum orientale* and other species of *Pedalineæ.* These seeds are white or black, the white seeds yielding the best oil. The plant is grown in India, China, Japan, Asia Minor, Java, Brazil and Mexico. The highest grade oil comes from the Levant. The seeds contain 50–57% of oil, the yield on expression being from 42–48%.

Sesamé oil is pale yellow in colour, deposits very little "stearine" on standing, and has a pleasant nutty flavour. Adulteration is sometimes practised with poppyseed oil (which raises the iodine value), cottonseed oil (raising the titer), rape oil (lowering the saponification value), and arachis oil (Renard's test).

A special colour test, sensitive to 1% of sesamé oil, is known as the Baudouin test. To 10 c.c. of the melted fat or liquid oil in a test-tube, add two drops of a 2% solution of furfural in alcohol, and 10 c.c. of concentrated HCl. After vigorous shaking the development of a *crimson* colour will indicate the presence of sesamé oil. On account of the delicacy and rapidity of this test, sesamé oil is a legal ear-marking ingredient in margarines on the Continent. Certain artificial colouring matters in margarines may seriously interfere with the test, when a special procedure is then necessary (see p. 142).

According to Lane,[1] the fatty acids obtained from sesamé oil are 12% solid acids, 16% linolic acid, and 72% oleic acid. The solid acids include stearic, palmitic and myristic acids.

Safflower Oil.—This oil, obtained from the seeds of *Carthamus tinctorius*, growing in India, China, Egypt and the Levant, is a golden-yellow coloured product of but slight odour and taste. In India the oil serves for edible purposes, and with the extension of the margarine industry there high-grade safflower oil will, no doubt, be utilized. Unfortunately the oil has a marked tendency to rancidity.

Shea-nut Oil.—Shea-nut oil or shea butter is expressed from the seeds of *Butyrospermum Parkii*, growing extensively in West and West-Central Africa, especially in Northern Nigeria.[2] The plum-shaped fruit ripens from May to September and encloses usually one, and sometimes two or three nuts, about 1½" long and 1" in diameter. The nut contains 50–60% of fat, which can be so refined as to yield a white and practically odourless and tasteless product. The consistency is similar to a high-melting lard, being a granular plastic solid.

In Africa the fat is used for edible purposes, and its use in Europe for margarines and for lard substitutes is increasingly important.[2] Trade names include karité butter, galam butter, and bambuk butter.

About one-third of the total fatty acids is stearic acid.[3] The

[1] *J.S.C.I.*, **13**, 69 (1894).
[2] Newland, *Coconuts, Kernels, Cacao* (London, 1919), Chap. IV.
[3] Southcombe, *J.S.C.I.*, **28**, 499 (1909).

fat contains from 5–9 per cent. of unsaponifiable matter, has a low saponification value, and an unusually high refractive index. Crystals obtained from ether solution very closely resemble the crystals given by beef fat. The fat may be fractionated to yield a "stearine" and an "oleine," the former being a substitute for cocoa-butter. The "oleine" is used in making lard substitutes and cooking fats, as well as in margarine.

Soya Bean Oil.—This oil, also known as soja oil or bean oil, is obtained from the seeds of *Glycine hispida* and varieties, grown extensively in China, Manchuria, Japan, Formosa, Korea and Indo-China. The bean is a little smaller than a pea, and is of a pale yellow colour. It contains about 18% of oil, of which about 13% is the commercial yield. Expression in modern mills with Anglo-American presses only yields 10% of oil. A large amount of soya oil is also obtained by solvent extraction, naphtha being the usual solvent employed.

Although the beans were not introduced into England until 1908, soya oil has found a ready and increasing use in margarines and lard-substitutes. The refined oil is admirably suited for edible purposes. When obtained by pressing, the oil has a brownish-yellow colour, whilst the extracted oil is a pale yellow. It is liquid at ordinary temperatures, and does not yield a "stearine" deposit. The taste and smell are but slight, and are quite pleasant. In America "prime soya bean oil" must be pressed, not extracted, and should have a colour not exceeding 35 yellow and 9 red, on Lovibond's scale.

No special tests exist for soya oil, and the only likely adulterants are cottonseed oil (detected by the Halphen reaction) and linseed oil (which would raise the iodine and insoluble bromide values). The chemical composition as determined by Matthes and Dahle[1] showed 56% oleic acid, 19% linolic acid, 15% palmitic acid, and about 4·8% linolenic acid.

Sunflower Oil.—This oil is obtained from the seeds of the Common Sunflower, *Helianthus annuus*, cultivated in Russia, Hungary, India, China, Mexico and South Africa. The seeds contain from 45–53% of oil, which, when refined, is of a clear golden-yellow colour, and liquid at ordinary temperatures. It possesses but a slight odour and taste, both pleasant. No special tests are described for sunflower oil. In Europe the oil finds an extensive use as a salad oil and also for margarine

[1] *Archiv. d. Pharm.* (1911), 249, 424.

manufacture. It is expected that it will be more largely utilized in future as a constituent of margarine.

Tea-seed Oil.—This oil is obtained from the seeds of *Thea sasanqua*, Nois (Thunb.), a shrub related to the ordinary tea plant. China furnishes the most oil, which can be refined to yield an edible product of golden-yellow colour, and very low, free, fatty acid content, *e. g.* 0·2% (oleic acid). The pressed oil contains saponin which is physiologically harmful. Extracted oil is considered free from saponin. Tea-seed oil has only been recently used in margarine manufacture, but there is no reason why a saponin-free product of high grade should be discountenanced.

Miscellaneous Oils and Fats.—Recently numerous palm and palm kernel oils have been described which future exploitation may make available for use in margarine manufacture. Bolton and Hewer[1] describe several Brazilian fats closely allied to coconut or palm kernel oils. Those suitable for margarine include *Elœis guineensis*, *Astrocaryum vulgare*, *Astrocaryum species*, *Acrocomia scelerocarpa*, *Maximiliana regia* (Anaja or kokerite palm), *Cocos syagrus* and *Attalea funifera* (which yields babassu kernel oil).

Newland[2] describes several possible sources of fats suitable for margarine, including fat from the kernels of Tucan nuts, Paraguay nuts (very similar to the West Indian gru-gru kernels of *Acrocomia scelerocarpa*), Strephonema nuts (the Belgian Congo), N'kula nuts (of *Coula edulis*), and the Kamoot nut of Sierra Leone. The following table shows the analytical constants of Tucan kernel oil and Paraguay kernel oil—

	Tucan.	Paraguay.
Melting-point	30·5° C.	—
Titer	27·0° C.	21·0° C.
Specific gravity $\frac{100° \text{ C.}}{15° \text{ C.}}$	0·867	0·867
Acid value	2·9	26·1
Saponification value	249	247
Iodine value (Hübl, 17 hours)	11·6 %	28·5%
Unsaponifiable matter	0·3 %	0·3%
Volatile acids, soluble	3·8	6·5
,, ,, insoluble	5·9	10·2

Another fat which may eventually find extensive use in

[1] *Analyst*, **42**, 35 (1917).
[2] *Coconuts, Kernels, Cacao* (London, 1919), Chap. VII.

margarine manufacture is the Indian Mowra fat,[1] obtained from the seeds of *Bassia latifolia and Bassia longifolia.* Mowra fat is an admixture of latifolia fat and longifolia fat (illipi fat), and is a soft, pale yellow product, plastic and somewhat softer than butter. It possesses a pleasant odour and flavour.

India also furnishes phulwa fat, obtained from *Bassia butyracea.* This fat is white, firm and somewhat stiffer than lard, and possesses a pleasant odour and taste. So far the kernels of *B. butyracea* are only used in India in preparing edible fats, but undoubtedly they would find a ready market in Europe.

The exploitation of tropical sources of fats suitable for margarine will undoubtedly lead to the future employment of many fats closely resembling either palm oil, palm kernel oil, or coconut oil.

[1] Dunstan, *Oil Seeds and Feeding Cakes* (London, 1917), Chap. V. Bolton and Revis, *Fatty Foods* (London), pp. 192–5.

CHAPTER III

EDIBLE HYDROGENATED OILS

Of recent years a new source of edible fat has appeared in the form of "hardened" or "hydrogenated" oils, which already exercises a marked influence on margarine manufacture. By suitable treatment with hydrogen in the presence of catalytic agents, liquid oils may be transformed into hard fats of various melting-points, the hydrogen being absorbed by the unsaturated fatty acid constituents of the oils. Thus, if cottonseed oil be treated with hydrogen under suitable conditions of temperature, pressure, and nature of catalyst, a series of products may be obtained depending on the amount of the gas absorbed. This combination between the oil and hydrogen has two main effects: (1) The iodine value of the oil decreases, and (2) the melting-point of the product increases. Thus, Moore, Richter and Arsdel[1] noted the following results when hydrogenating cotton-seed oil:—

Melting-point in °C. .	9·0	39·4	40·8	45·8	48·0	48·9	60·5
Iodine No. . . .	107	74·3	66·7	61·0	54·5	48·5	0·4

A great number of patents have been granted for conducting such oil-hardening, wherein the main idea sought is to obtain an intimate contact between the hydrogen gas, the oil, and the catalytic agent, which is often nickel in very fine and pure form, or its oxide or salts. Other catalysts proposed are palladium, platinum, copper, iron and other metals. The use of catalysts in colloidal suspension or solution is also claimed. The subject-matter of the many patents does not concern us here, but the following are some of the most notable patents granted in connection with edible-oil-hardening:—[2]

Bedford and Williams (U.S. 1026339/12); Boyce (U.S. 1061254/13); Calvert (Eng. 18350/13); Ellis (U.S. 1026156/12); Ellis (1095144/14); Erdmann (Ger. 211669/07); Kayser (U.S. 1004035/11); Lessing (Eng. 18998/12); Maxted and Ridsdale (Eng. 109993/17); Schlink & Co. (Eng. 8147/11); Shukoff (Ger.

[1] *J. Ind. Eng. Chem.*, **9**, 451–62 (1917).
[2] Cf. Ellis, *Hydrogenation of Oils* (Constable, London, 1914).

241823/10); Testrup (Eng. 7726/10); Wilbuschewitsch (Eng. 30014/10).

Hydrogenation may be stopped at any desired stage, and the properties of hydrogenated oils therefore depend on the degree of hardening attained. The analytical constants of the original oil undergo a change, except in the case of the acid value, saponification value, amount of unsaponifiable matter, Reichert-Meissl value, and Polenské value, which are practically unaffected. The iodine number decreases in proportion to the amount of hydrogen taken up. The specific gravity and the melting-point increase, the former to the limit value for tristearin (1·0101 at 15° C.),[1] and the latter to a degree dependent on the molecular weight and the hydroxyl content of the fatty acid constituents of the oil. The refractive index and acetyl number decrease. The following results, due to Bomer,[2] indicate the changes in the usual constants of an oil when hydrogenated:—

Oil.	Appearance.	°C. melting-point.	Solidifying-point.	Refractive index at 40° C.	Acid No.	Saponification No.	Iodine No.
Arachis (untreated)	Yellow liquid	—	—	56·8	1·1	191·1	84·4
,, (hardened)	White tallowy	51·2	36·5	50·1	1·0	188·7	47·4
Coconut (untreated)	White soft	25·6	20·4	37·4	0·3	255·6	11·8
,, (hardened)	Like lard	44·5	27·7	35·9	0·4	254·1	1·0

After "hardening" certain specific tests for individual oils become inoperative. Thus the elaïdin test is no longer applicable; the Halphen test for cottonseed oil and the Becchi test also fail, though the Baudouin test for sesamé oil is uninfluenced. Hardened fish oils no longer retain their essential characteristics, such as the formation of well-defined bromine compounds of the higher unsaturated fatty acids. Grimme[3] has attempted to identify fish oils after hydrogenating by means of colour tests, whereby he distinguishes between seal, whale, liver, and fish oils. Thus, if 5 parts of hardened whale oil be dissolved in 95 parts of benzine-xylene (1 : 1), and 5 c.cs. be shaken with 1 c.c. of concentrated sulphuric acid and 1 drop of tincture of iodine, a characteristic violet-red colour is produced in the fatty layer.

Although contrary opinions have been expressed, it is now

[1] Normann and Hugel, *Chem. Zeit.* (1913), p. 815.
[2] *Chem. Rev. u. d. Fett. Harz. Ind.* (1912), p. 220.
[3] *Ibid.*, (1913) pp. 129, 155.

generally agreed that during the hydrogenation of oils, cholesterol and phytosterol, the characteristic aromatic alcohols, indicating animal and vegetable origin respectively, are unaffected, and consequently the animal or vegetable origin of a hardened oil may still be ascertained. Hardened oils are usually examined for the presence of nickel, the catalyst most frequently employed, but there are now many hardened oils which do not show traces of nickel, indicating that some other catalyst has been used, or that all traces of catalyst have been successfully removed. A most sensitive test for nickel, capable of detecting 1 part of this metal in 5 million of water, has been described by Atack:—[1]

A large sample of the fat is digested in a flask with an equal mass of pure concentrated hydrochloric acid for one to two hours on the water-bath, with frequent shaking. The mixture is transferred to a separating funnel, and the acid layer run off into a porcelain dish, which is then completely evaporated on the water-bath. The residue is dissolved in 50 c.cs. of hot absolute alcohol, made slightly alkaline with ammonia, and 50 c.cs. of a hot saturated solution of α-benzildioxime in absolute alcohol are added. A red precipitate is produced in the presence of nickel. After a few minutes heating, the precipitate is collected on a tared filter-paper, washed with hot alcohol, dried at 110° C. and weighed. The weight of the precipitate multiplied by the factor 0·1093 gives the weight of nickel present.

Hardened oils may be prepared which very closely resemble beef, mutton tallow or lard in colour and consistency. Whale oil has been extensively hardened for use in margarine manufacture, particularly as a substitute for beef-stearin in cheaper grades. Sandelin[2] examined several such fats prepared in Germany and obtained the following figures:—

	Melting-point.	Solidifying-point.	Acid value.	Saponification value.	Iodine number.	Reichert-Meissl value.	Molar. weight of insoluble fatty acids.	Melting-point of the arachidic acid.
Original whale oil	Fluid	Fluid	9·50	192·2	144·8	0·27	287·7	—
Artificial tallow .	47·5° C.	38·1° C.	9·88	183·7	56·9	0·25	296·4	75·5° C.
Artificial stearine	54·3° C.	47·3° C.	7·80	187·7	11·7	0·14	297·0	74·1 C.
Hydrogenated whale oil	41·9° C.	31·9° C.	5·30	190·9	57·8	0·18	282·0	76·0 C.

[1] *Chem. Zeit.*, **37**, 773 (1913). [2] *J. Soc. Chem. Ind.*, **33**, 1097 (1914).

Samples of Japanese hardened whale oil intended as a substitute for beef-stearin, have yielded the following analyses in the author's laboratory :—

Melting-point. °C.	% F.F.A. as oleïc.	Saponification value.	Iodine number.	Reichert-Meissl value.	Polenské value.
39·0	0·28	176·4	55·7	—	—
50·0	0·18	162·4	27·2	—	—
40·5	0·06	202·8	64·6	1·24	1·14
50·0	2·48	201·0	40·8	1·05	—
46·0	0·37	201·4	40·6	—	—
48·0	2·03	201·7	30·8	—	—
49·0	2·78	203·2	38·6	—	—
46·0	1·72	204·6	28·7	—	—
49·0	2·46	197·7	30·3	—	—
51·0	2·48	199·8	31·8	—	—
36·0	4·82	206·2	66·15	0·22	0·60
42·5	1·20	203·2	83·66	0·58	0·74

Apart from their use in margarine manufacture, hardened oils have a more extensive use in the making of lard substitutes, *i. e.* "lard compound." There are two main modes of making such products: (1) The "compound" is made by the "self-thickening" of a liquid oil such as cottonseed oil; or (2) hardened oil of suitable melting-point is incorporated with the requisite amount of cottonseed (or other) oil. In the first process, the oil is hydrogenated to just such a degree as to yield a product of lard-like consistency. In the second, a hard fat is melted in the vegetable oil, and suddenly chilled, yielding a white product of lard-like nature. See p. 130 for fuller details.

The chief oils hardened for edible purposes are soya, cotton-seed, rape, arachis, sesamé, linseed and sunflower oils, and whale, seal, and other fish oils. Their keeping qualities are excellent; thus Knapp [1] reports on samples of hardened oils kept for nearly eighteen months, often exposed to damp air. The free fatty acids (0·7% as oleic acid) had not appreciably increased in amount. Usually, hardened oils are of excellent colour, and are free from taste and smell. From time to time, hardened oils are offered for edible purposes which possess a very characteristic burnt odour, comparable with that of distilled olein, which is particularly penetrating in any margarine or lard-compound made therefrom. Apparently hydrogenation at high temperatures leads to a decomposition of the oil, whereby acrolein of a most pungent odour is produced.

[1] *Analyst*, **38**, 102 (1913).

Formerly, a strong objection to the edibility of hydrogenated oils was made because of the nickel likely to be present. Bomer[1] found that neutral oils are readily freed from nickel, but when the free fatty acids are in considerable amount, notable quantities of nickel are dissolved and retained in a form extremely difficult to remove. Thus hardened sesamé oil of a free acidity of 2·5% oleic acid gave an ash of 0·01% containing 0·006% nickel oxide; a whale oil of which the acidity was 0·6% as oleic acid gave an ash of 0·006% containing 0·0045% nickel oxide.

It is now generally agreed that nickel in the amounts likely to be present in hardened oils has no detrimental physiological action. Offerdahl[2] observed that hardened whale oil contained on the average 0·5–2 milligrams of nickel per kilogram. In experiments to determine its physiological action, as much as 0·5 gram of nickel oxide powder was taken daily, and no ill-effects observed; in fact, 99·8% of the metal was rapidly eliminated from the system in the excreta. Physiological tests conducted with hydrogenated cottonseed, arachis, and sesamé oils, showed they were perfectly wholesome and that 1–2 milligrams of nickel per kilogram of body weight were quite harmless.[3] Experiments on a large scale in an institution were carried out by Erlandsen, Fridricia, and Elgström in 1918.[4] Two hundred and fifty people were fed with a margarine containing 22·8% of a hydrogenated whale oil, but no deleterious results were observed by the attendant medical officer. It was further shown that the whale-fat was almost as completely digested as butter-fat, the apparent digestibility of the two fats ranging from 91·6% to 94·9%, with a maximum difference between the whale-fat and butter-fat figures of 0·9%.

Similar results were obtained by Thomas and Müller,[5] who used hydrogenated arachis, cottonseed and pine oils; extensive chemical and physiological tests convinced them that hydrogenated oils were very useful and entirely harmless in the human diet. Lehmann,[6] as a result of feeding hardened arachis, sesamé, and cottonseed oils to dogs and human beings, to the extent of 7 lb. of such fat per month for periods ranging from two to four months, concludes that they form a " thoroughly satisfactory

[1] *Chem. Rev. Fett. Ind.*, **19**, 221 (1912).
[2] *Ber. deutsch. pharm. Ges.*, **23**, 558 (1913).
[3] *Monats. für Margarin Ind.; Seifenfabr.*, **34**, 181 (1914).
[4] *Tidskrift Kem.*, **15**, 109–33 (1918).
[5] *Arch. Hyg.*, **84**, 56–77 (1915). [6] *Chem. Zeit.*, **38**, 798 (1914).

food." This conclusion is further supported by the work of Pekelharing and Schut [1] on hardened whale, cottonseed, sesamé and arachis oils, with, however, this qualification, that such fats should not be the sole fatty diet of man, but are best mixed with some natural fat, such as lard.

The most recent authoritative statements on this matter are equally satisfactory. Thus Fahrion [2] is convinced that hardened oils, including hydrogenated marine animal oils, possess no hygienic drawback whatever, and are excellent foods. In a report furnished to the French Minister of the Interior, Bordas also concludes that hydrogenated oils are quite suitable for edible purposes. As regards their nickel content, they contained less nickel than other common foods cooked in nickel vessels, and in any case, he found that so large a daily dose of nickel salt as to correspond to 0·5 gram of nickel, continued for fifty-two days, was entirely without injurious effect.[3] The fact that everyday foods cooked in nickel vessels contained fully as much nickel as any hardened oil has been conclusively demonstrated by Ludwig,[4] Lehmann,[5] and by Normann and Hugel.[6]

Recent investigations on the nutritional chemistry of foods in general have shown that hydrogenated oils are totally deficient in a most important substance, known as the " accessory food substance, fat-soluble A." This substance, which plays a profound *rôle* in animal nutrition, is apparently absent in vegetable oils and fats, and consequently in their hydrogenated products, and it seems to be destroyed during the hydrogenation of animal oils and fats.[7]

Despite this objection, it must be admitted that the introduction of hardened oils marks a distinct advance in margarine technology, and in the manufacture of lard substitutes. In the latter case, the absence of the " fat-soluble A " in hardened oils is no immediate detriment, since natural lard itself is exceptional amongst animal fats in being wholly deficient in " fat-soluble A." An abundant source of clean, wholesome and reliable raw material is now available in addition to the usual animal and vegetable oils and fats hitherto solely employed. In time, a

[1] *Pharm. Weekblad.*, **53**, 769–85 (1916).
[2] *Chem. Umschau.*, **26**, 22, 33 (1919).
[3] *Ann. Falsif.*, **12**, 225 (1919).
[4] *Osterr. Chem. Zeit. Vienna*, Vol. I. No. 1 (1898).
[5] *Arch. Hyg.*, **68**, 421 (1909).
[6] *Seifen Zeit.* (1913), p. 959.
[7] *J. Physiology*, **51**, 235 (1917); *Biochemical Journal*, **13**, 81 (1919).

simple margarine may be manufactured, consisting of a single highly-refined edible oil hydrogenated to the desired butter consistency; the nutritive element of fat-soluble accessory food substance being introduced either by blending with butter already containing it, or by the addition of a synthetic fat-soluble A resulting from biochemical research.

CHAPTER IV

THE EXAMINATION OF MILK FOR USE IN MARGARINE MANUFACTURE

As milk is so important a constituent of almost every margarine, and is itself so liable to foreign infection which would militate against its flavouring properties in the finished margarine, a most careful chemical and bacteriological control must be regularly exercised. Only clean, freshly-separated milk of undoubted quality should be employed, in order to ensure a desirable flavour and good keeping qualities in the finished product.

The routine chemical analysis of the milk includes determinations of total solids, fat, acidity, and the detection of possible preservatives. The bacteriological tests ascertain the cleanliness, age, and previous treatment of the milk (*e. g.* pasteurization), and enable a definite opinion to be made as to its suitability for margarine manufacture. Naturally, bacteriological tests which need considerable time for their carrying out cannot be employed, as the milk is usually handled immediately on arrival at the factory. Such tests are useful as confirmatory tests in cases of doubt or dispute.

Chemical Control

Total Solids.—The total solids may be rapidly determined by the simple evaporation of the milk to constant weight: 5 grams of milk are weighed into a shallow dish (preferably of platinum or quartz) or into a glazed-porcelain "milk-testing dish," which is $2\frac{3}{4}$" in diameter, flat-bottomed, and $\frac{1}{2}$–$\frac{3}{4}$" deep. 1 c.c. of acetone is added and the dish placed on a water-bath for thirty minutes, and the residue dried to constant weight in the steam oven, which requires about one hour. The increase in weight of the dish, multiplied by 20, indicates the percentage of total solids present in the milk. The average percentage of total solids in full-cream milk is 12%, and in separated milk 8·5%.

Fat.—The fat is estimated volumetrically, employing mechanical separation, as in the Babcock, Leffmann-Beam and Gerber methods. For control work in margarine factories, the Gerber method is usually employed. The apparatus necessary comprises a butyrometer and a centrifugal-separating machine worked at 2000 revolutions per minute. 10 c.cs. of sulphuric acid (sp. gr. 1·820–1·825) are run into the butyrometer, 11 c.cs. of the milk to be tested carefully added so as to avoid mixing with the acid, together with 1 c.c. of amyl alcohol (b.-pt. 124–130° C., sp. gr. 0·815–0·818). The butyrometer is then securely closed by an indiarubber stopper. The tube is well agitated with an up-and-down motion so as to effect solution of the curd, and heated in a thermostat at 70° C. for a quarter of an hour. It is then placed in a centrifuge for five minutes, when the fat will have collected in the specially-graduated neck of the butyrometer, and its volume is read off in direct percentages.

Acidity.—The acidity is often expressed in terms of lactic acid, though it is preferable to employ an arbitrary scale of "*degrees of acidity.*" This is due to the fact that phosphates in milk largely contribute to the acidity of fresh milk. The "degrees of acidity" of a sample indicate the number of cubic centimetres of $\frac{N}{1}$ alkali required to neutralize 1 litre of milk. To carry out the estimation, 20 c.cs. of milk are titrated with $\frac{N}{10}$ NaOH until just pink to phenolphthalein (5 drops of a 1% solution). Then, the number of cubic centimeters of the alkali required, multiplied by 5, gives the acidity in degrees. If the acidity is required in terms of lactic acid, 1 c.c. of $\frac{N}{10}$ NaOH is taken as being equivalent to 0·009 gram lactic acid.

Fresh clean milk has an acidity of 15–18°, whereas milk which is sufficiently sour to just curdle on heating reaches 24°. Milk "ripened" for margarine churning contains free lactic acid, and consequently its acidity is usually reckoned in terms of *percentage of lactic acid.*

Preservatives.—The usual preservatives found in milk are boron compounds and formaldehyde.

To test for *boron compounds,* to 10 c.cs. of milk add 3 c.cs. of methyl alcohol, and 5 drops of a saturated alcoholic solution of turmeric. Heat on a water-bath, when, if boron compounds be present, a *salmon-pink* ring will form around the edge of the dish.

To test for *formaldehyde* the Shrewsbury and Knapp[1] test is both trustworthy and delicate: 5 c.cs. of milk are mixed in a test-tube with 10 c.cs. of pure, strong HCl containing 0·1% of pure HNO_3. The mixture is heated to 50° C. for ten minutes on a water-bath, and then cooled to 15° C. If formaldehyde is present, even as little as 0·2 part per million, a violet coloration is produced, whilst in the absence of formaldehyde a rose-pink colour ensues.

Benzoic and Salicylic Acids.—These substances are very seldom used, but may be detected by precipitating the curd by dilute mineral acid from the clear milk serum, and extracting the acids by ether. The ether extract is shaken with water, made alkaline with ammonia, and the aqueous layer evaporated almost to dryness. A few drops of neutral and freshly-prepared ferric chloride solution are added, when a buff-coloured precipitate will indicate benzoic acid, and a violet coloration salicylic acid.

Bacteriological Control

Cleanliness of the Milk.—Good fresh milk should show no appreciable sediment on standing. To ascertain its freedom from sediment, 100 c.cs. of the sample are placed in a V-shaped glass, and after an hour or two any sediment is carefully drawn up into a pipette. This sediment is stirred in 50 c.cs. of water and again allowed to stand an hour, after which a sample of the deposit is withdrawn by means of a capillary pipette and examined under a microscope using a $\frac{1}{4}''$ objective. Particles of straw, sand, fæces, and other foreign bodies are easily detected. If the slide is now dried, immersed in 1% aqueous methylene-blue for a minute, and then washed in water, an examination under the microscope, using the $\frac{1}{12}''$ oil-immersion lens, will detect the presence of cells characteristic of milk given by cows suffering from mastitis. Such cells must be present in abnormally large numbers before an adverse opinion is given as to the hygienic value of the milk. A careful investigation carried out for the New York Milk Committee on this question showed that: "no method has yet been accepted for accurately distinguishing between the pus cells and other cells that may be in the milk that do not have an origin in inflammatory conditions. . . . A general consensus of opinion has been reached

[1] *Analyst*, **34**, 12 (1909).

that a high cell count should not alone condemn milk, although it is a matter for suspicion. The cell count varies with different cows on different days." [1]

Some authorities believe that a high cell count accompanied by streptococci is a sure indication of udder disease.

Bacillus Enteritidis Sporogenes Test.—This test affords valuable information as to pollution by cow-dung, etc., in the *original* milk, since the spores of *B. enteritidis sporogenes*, so abundant in manure, do not multiply in the milk. Dirty containers will also contaminate milk with these spores.

To examine the milk for this form of pollution 10 c.cs. of milk are run from a sterilized pipette into a sterilized test-tube, and ¾" of melted vaseline poured on to the surface. The tube is plugged with sterilized cotton-wool, singed just before insertion, and then heated to 80° C. for fifteen minutes in a water-bath. After subsequent cooling, the milk is incubated at 37·5° C. under anærobic conditions for twenty-four hours. Dirty milk, containing spores of *B. enteritidis sporogenes* (which can survive 80° C.), shows a very characteristic change, the "*enteritidis* change," in which peptonization of the casein occurs and more or less gas is evolved. The curd appears "blown" and stringy, the vaseline seal is displaced, and the odour of butyric acid is pronounced. Examination of a portion of the curd when stained shows under the microscope thick bacilli with rounded ends.

Tests for Pasteurized Milk.—In margarine practice milk is examined on arrival for evidence of previous pasteurizing treatment, and the usual method of such treatment necessary in the dairy department before souring with lactic acid cultures also needs control tests. Many methods have been proposed to distinguish between raw and heated milks; for full-cream milk a determination of the cream-line is simple and definite, whilst for separated milk tests based on enzyme reactions are usual.

Cream-line Test.—100 c.cs. of milk are allowed to stand for six hours in a graduated cylinder at 15·5° C. For each 1% of fat in the initial milk sample there should now be apparent 2·5% of cream. If less than this is given, the milk may be assumed to have been pasteurized at a temperature above 65° C., or to be a mixture of new and sterilised milk.[2]

Benzidene Acetate Test.—This test indicates not only whether

[1] Public Health Department, Washington. Report No. **78** (1912).
[2] *Examination of Milk for Public Health Purposes*, by Race (New York, 1918), p. 91.

milk has been heated or not, but also to what temperature it was raised and for what length of time. The test is best conducted as follows:[1] To 10 c.cs. of milk add 1 c.cs. of a 10% solution of benzidene in 96% alcohol, 3 drops of 30% acetic acid, and finally, 2 c.cs. of 3% hydrogen peroxide. Peroxidase, present in raw milk and destroyed in heated milk, produces a deep azure-blue colour, particularly pronounced in the curd which is precipitated. The following table summarizes the results obtained by Race on the effect of heat on the peroxidase test.

Duration of heating in minutes.	Benzidene reaction after heating to:—					
	63° C.	65·5° C.	68·5° C.	71° C.	74° C.	76° C.
5	+	+	+	+	+	+
10	+	+	+	+	+	+
15	+	+	+	+	+	Faint
20	+	+	+	+	Faint	Very faint
25	+	+	+	Faint	—	—
30	+	+	+	Very faint	—	—

The + sign indicates the appearance of the blue colour, showing that the milk has not been completely pasteurized.

Two kinds of pasteurizing processes are known, depending on whether the milk has been passed in a continuous stream through a heating device at about 82° C. ("flash" pasteurization), or whether the milk has been contained in one vessel and heated gradually to about 70° C. for thirty to forty minutes ("bulk" or "holder" pasteurization). A means of differentiating between milks pasteurized by these two methods consists in fermenting the milk at 38–39° C. About 50 c.cs. of the milk are placed in a sterilized boiling-tube (6″ × 1¼″) fitted with a metal cover. After incubating at 38–39° C. for twelve hours, the milk is examined to determine the extent and nature of curdling. "Bulk" pasteurized milk will either not have curdled at all, or will have set to a fine, homogeneous, gelatinous curd, free from gas bubbles, whereas milk heated to above 80° C. will have undergone peptonization, since only spore-formers develop, and being unchecked by lactic organisms, rapidly increase, producing fermenting reagents which induce precipitation and digestion of the caseinogen.

The Reductase Test.—A fairly conclusive idea as to the

[1] *Loc. cit.*, p. 188.

number of micro-organisms present in milk, and thus as to the age of the milk, is given by following the course of the reduction of methylene blue by a standard volume of milk at 38–39° C. Chiefly from the work of Schardinger [1] it has become recognized that two classes of *reductases* may be present in milk: (1) A reductase of cellular origin, enzymic in character, which rapidly decolorizes methylene blue in the presence of a little formaldehyde. This enzyme is present in fresh milk, and is destroyed by heating, and consequently is absent in pasteurized milk; (2) a reductase of bacterial origin, capable of decolorizing methylene blue in the absence of formaldehyde. Both reductases are sharply distinguished from another enzyme, catalase, in that, while all three abstract oxygen from suitable compounds, there is no liberation of free (gaseous) oxygen by the reductases, whilst in the case of catalase, the liberated oxygen may be volumetrically estimated.

The test as carried out by Barthel and Orla-Jensen [2] is as follows: 40 c.cs. of milk are poured into a clean sterilized tube, 19 cms. × 2 cms., marked at 40 c.cs. After adding 1 c.c. of a 0·015% aqueous solution of methylene blue (standardized against titanous chloride [3]) and shaking, the tube is covered with a metal cap and immersed in a thermostat at 38–39° C. The time required for complete decolorization is noted. (In the case of separated milk, re-oxidation of the surface layer may be prevented by covering with a little paraffin or liquid vaseline.) Barthel and Orla-Jensen classify milk by this test as follows:—

Class.	Time required to decolorize.	Number of organisms per c.c.
1	5½ hours or longer	Less than ½ million
2	From 2 to 5½ hours	½ to 4 millions
3	From 20 minutes to 2 hours	4 to 20 millions
4	Less than 20 minutes	Over 20 millions

A critical study of this test by Arup [4] leads to the conclusion that a lower temperature (28–29° C.) of incubation is preferable to 38–39° C. This is particularly the case if the test is to be applied to milk known to have been pasteurized before delivery to the factory. "Comparison with the results obtained with

[1] *Z. Unter. Nahrs. Genuss.*, **5**, 1113–21 (1909).
[2] *Milchwirts. Zentralb.* (1912), p. 14.
[3] Knecht and Hibbert, *New Reduction Methods in Volumetric Analysis.*
[4] *Analyst.* 43, 20–31 (1918).

raw milk brings out the fact that the organisms surviving in pasteurized milk appear to reduce more rapidly than those of raw milk, the effect being more pronounced the higher the temperature to which the milk has been subjected. Considerable bacterial reduction may take place without any correspondingly great increase in the reduction time; the sensitiveness of the test is diminished in such cases. If the raw milk was not bad, inefficient pasteurization is not detected, and all that can be said is that a standard of at least nine hours might reasonably be fixed for newly-pasteurized milk. This period of time should be increased if the test is carried out at a lower temperature" (Arup., *loc. cit.*).

For use in margarine manufacture, milk subjected to this test should still be coloured blue even after six hours' incubation.

CHAPTER V

THE MANUFACTURE OF MARGARINE

Preparation of the Fatty Portion.—The first stage in margarine manufacture is the melting of the fatty ingredients. The composition of the fatty portion varies with the quality of the margarine, the time of the year, and the prevailing market conditions. For ordinary table use, two classes of margarine are mainly made: (1) oleomargarine, in which animal fats form the major portion, and (2) vegetable margarine containing vegetable fats such as coconut oil, as the essential ingredients. The well-known "nuts and milk margarines" come under this class. The mixture of oils and fats is so arranged as to possess a melting-point from 22–27° C. The composition of a margarine of either class admits of considerable variation, but the following formulæ are typical and have been extensively employed in actual manufacture.

Oleomargarine.—During the war the following formula was officially proposed (May 1918) as a standard for a 55% animal fat margarine—

	%
Neutral lard	5
Premier Jus	0
Oleo	30
Coconut oil	20
Cottonseed oil	
	100

Of other formulæ employed in English factories in pre-war days, the following may be cited—

A	%	*B*	%	*C*	%
Neutral lard	29	Neutral lard	20	Neutral lard	16
Oleo	60	Oleo	55	Oleo	50
Arachis oil	11	Coconut oil	11	Coconut oil	13
	100	Arachis oil	14	Premier Jus	6
			100	Arachis oil	15
					100

The melting-points would be about 27·6° C., 26·0° C., and 26·0° C. respectively. Instead of arachis oil, the best-refined cottonseed oil is frequently substituted, and indeed there is little to choose between them.

Vegetable Margarines.—The officially-proposed war-formula (May 1918) was—

	%
Premiser Jus	15
Coconut oil	15
Palm-kernel oil	50
Arachis oil	10
Cottonseed oil	10
	100

The melting-point is about 25° C. The formula was altered to suit the time of the year, and in November 1918 contained 30% liquid oils, 20% animal fats, and 50% vegetable fats. A good vegetable margarine may be made from 85% of coconut oil and 15% liquid oil; or from 30% palm kernel oil with 60% coconut oil and 10% liquid oil. Sometimes a hard fat such as stearine is incorporated, as for example in the following composition—

	%
Oleostearine	15
Coconut oil	45
Palm-kernel oil	20
Arachis oil	20
	100

Melting-point = 24·5° C.

In most Continental margarines, 10% of sesamé oil is a compulsory ingredient, whereby analytical detection of the product is assured. In the United States of America, where artificial colouring matters are forbidden, it is often the practice to add palm oil, mustard seed oil, or deeply tinted corn oil.

In addition to margarines for table use, there are also special qualities made for bakers and confectioners. The bakers' margarine has a low melting-point and must be easily beaten into a creamy paste. Instead of the highest grade arachis or cottonseed oils, such oils as soya, kapok, wheat, maize, and second-class arachis and cottonseed oils are employed; otherwise the formulæ of *cake* margarines, as they are called, approximate to those intended for table use. Samples of well-known brands of

cake margarines recently examined in the author's laboratory were found to correspond to the following compositions—

A	%	*B*	%	*C*	%
Oleostearine	23	Coconut oil	70	Premier Jus	37
Premier Jus	30	Palm-kernel oil	10	Oleo	21
Cottonseed oil	47	Cottonseed oil	20	Cottonseed oil	42
	100		100		100
M.-pt. = 24° C.		M.-pt. = 21·5° C.		M.-pt. = 24° C.	

Margarine intended for use in pastry-making is a tough product of considerably higher melting-point than the foregoing. Oleostearine is a frequent constituent, and "pastry" margarines have recently been made which contained up to 50% of hydrogenated fish oil (melting at 50° C.). Some typical formulæ are—

A	%	*B*	%	*C*	%
Oleostearine	65	Oleostearine	50	Oleostearine	35
Premier Jus	15	Premier Jus	30	Premier Jus	20
Liquid oils	20	Liquid oils	20	Palm-kernel oil	35
				Liquid oils	10
	100		100		100
M.-pt. = 40° C.		M.-pt. = 39° C.		M.-pt. = 37° C.	

Though table margarine is always churned with milk, it is optional to do so with "cake" margarine, and it is seldom the practice with "pastry" margarine.

The composition having been decided upon, the solid fats are first melted, and then the liquid oils run in, and the whole intimately mixed. The melting-pans are double-jacketed, so as to allow of steam-heating, and, if required, of water-cooling, and are fitted with agitating apparatus. Instead of separate pans, batteries of six or more are coming into favour (see Fig. 1). For other types of melting-pans reference may be made to catalogues of margarine machinery. When melting is complete, artificial colouring matter is added and the well-stirred mixture cooled to the desired temperature, depending upon the composition and the temperature required for the subsequent emulsification, before emulsifying the fatty portion with the milk or water as the case may be.

The added colouring matter consists of an oil-soluble dye (see p. 88), and is usually a coal-tar product; annatto, the former

widely-used colouring principle, being seldom or never used. According to Lubs [1] 1 lb. of butter seldom contains more than 1 milligram of dyestuff, and 1 part in 100,000 is readily identified. Margarines also would seldom contain over 2 milligrams of dyestuff per 1 lb. The colouring matters may be bought already dissolved in oil, when from 1–1½ litres per ton of melted fats is

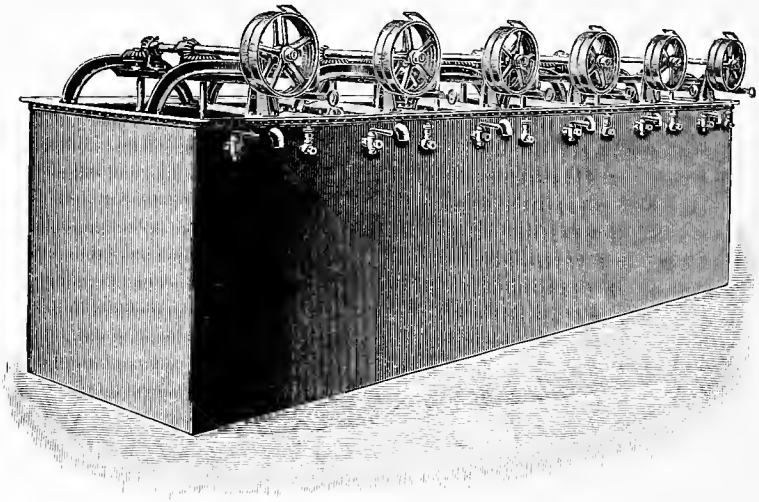

FIG. 1.—BATTERY OF MELTING PANS.
(N.V. Grasso's Machinefabrieken).

quite sufficient for a medium colour to be given to the final product.

Before the melted fatty constituents are churned or emulsified with the milk, this ingredient has to be specially prepared, so as to impart the desired flavour to the margarine. The preparation includes destruction of undesirable micro-organisms (pasteurization), and subsequent souring with pure cultures of lactic acid-producing bacteria.

[1] *J. Ind. Eng. Chem.*, **10**, 436 (1918).

CHAPTER VI

THE MANUFACTURE OF MARGARINE (*continued*)

Preparation of the Milk.—Milk is employed in margarine manufacture primarily as a source of flavour, and secondly as an emulsifying agent.

The *pasteurization process* has for its object the destruction of all pathogenic bacteria and other micro-organisms inimical to a good flavour. The milk is thus left in a suitable condition for the growth of the desirable lactic acid bacteria, whose development causes acidity and curdling of the milk, which is then very similar in aroma and taste to freshly-made buttermilk. From a theoretical point of view, *sterilization* of the milk would be more preferable, whereby every type of organism present would be destroyed. However, this is not practicable, since to ensure sterilization the milk would have to be maintained at 100° C. for six hours,[1] or heated by means of steam under pressure, whereby the flavour would acquire a burnt taste, through the caramelization [2] of the lactose or milk-sugar.

Pasteurization, as applied to milk, is really a temporary preservation, by destroying certain classes of bacteria whose normal development would sooner or later cause the milk to become "bad." Soxhlet introduced the pasteurization of milk, and originally this meant heating to near the boiling-point and then rapidly cooling. At present, by the pasteurization of milk, is to be understood heating the liquid to between 60–68° C. for thirty minutes, and then rapidly cooling it. By this treatment about 92–99% of the total organisms present are destroyed, including all the pathogenic (disease-producing) types.

There are two main methods of effecting the pasteurization of milk: (1) the "flash" or "continuous" process, and (2) the "bulk," "holder," or "vat" method.

In the first process the milk is heated to 71° C. or higher for only thirty to sixty seconds, whilst in the second the milk is maintained

[1] Hewlett, *Manual of Bacteriology*, p. 614 (London, 1914).
[2] Ernst, *Milk Hygiene*, pp. 193–202 (Chicago, 1914).

at 60–65° C. for thirty minutes. The second is the more effective method and by far superior in all respects. From the bacteriological point of view the second method has the great advantage that all pathogenic bacteria are destroyed, as well as the peptonizing types, whilst the "flash" method leaves the peptonizing bacteria very little affected. Moreover, the chemical changes induced by high temperatures, such as occur in the "flash" process, viz. coagulation of albumin, and precipitation of the soluble calcium and magnesium phosphates, are absent in the second method, which thus constitutes a decided advantage. Rupp[1] showed that the higher the temperature to which milk was heated the greater the percentage of albumin coagulated. Thus—

Milk heated 30 mins. at—	% albumin precipitated.
62·8° C.	*nil*
65·6° C.	5·75
68·3° C.	12·75
71·0° C.	30·87

From the economic standpoint the "holder" process again has the advantage, since about 17% less heat is needed, and there is also a saving in the subsequent cooling back.

Pasteurizing Machinery

1. *Flash Process.*—Numerous machines have been devised,[2] one of the oldest and most efficient being the Danish heater. This apparatus is essentially a central cylinder, provided with a double jacket, which, when in use, is filled with water into which steam is blown at different parts. The milk either falls by gravity or is pumped into the machine, where, meeting high-speed revolving vanes, it is flung by centrifugal action against the heated surface of the inner jacket. Entering at the bottom of the cylinder, the milk is thrown towards an exit pipe at the top placed tangentially to the cylinder; a pump-like effect is thus produced, and, by setting the heater at a suitable angle, the pumping effect is increased, and the milk can be lifted many feet. Use is generally made of this fact to cause the pasteurized milk to flow down a vertical cooler (Fig. 2).

Regenerative-Plant.—It is becoming increasingly popular to employ the "regenerative principle" in pasteurization, *i. e.*

[1] *U.S. Dept. Agr. Bull.*, **166**, 1–15 (1913).

[2] For full description see Kilbourne, *Pasteurization of Milk* (Wiley, New York, 1916).

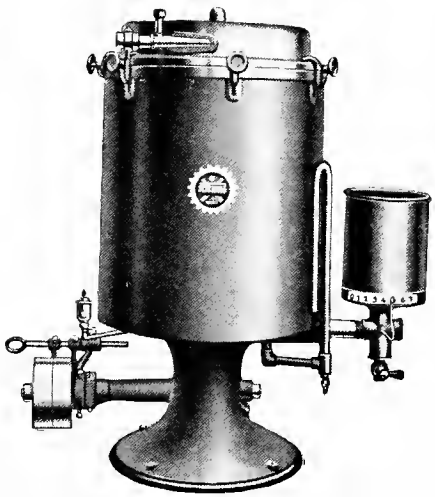

Fig. 2. "DANISH HEATER" PASTEURISER.

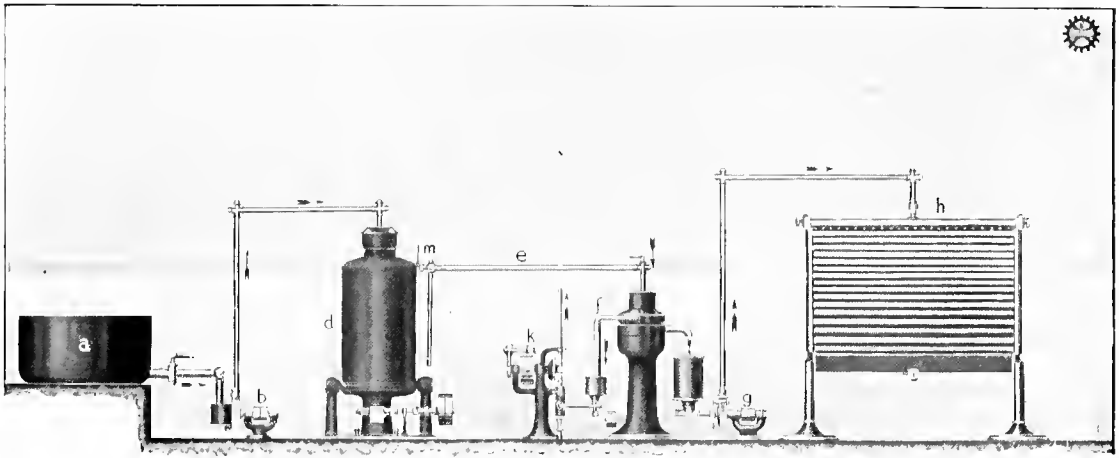

Fig. 3. REGENERATIVE PASTEURISING PLANT.

the hot pasteurized milk is used to warm up the incoming cold raw milk, and is itself cooled down in doing so. Economically this is a decided advantage, less steam being required for heating, and less ice-water for cooling. Exhaust steam may be utilized to advantage. The principle is well illustrated by Fig. 3, describing a regenerative-plant in conjunction with separated-milk-pasteurizing.

New milk flows from the tank (*a*), through a regulating funnel (*b*) which regulates the capacity and ensures an even flow to a centrifugal pump (*c*), whereby the milk is pumped over the regenerative heater (*d*), an even flow over the outside being secured.

Leaving the regenerative heater at the bottom, the milk passes to the separator (*e*) so as to remove the cream. From (*e*) the skim-milk discharges into the pasteurizer (*f*), whence, after the requisite heating, the hot milk passes to a centrifugal pump (*g*), which pumps the skim-milk upwards inside the regenerative heater (*d*). By this action the incoming whole milk is warmed up, absorbing heat from the pasteurized skim-milk, which is thus being cooled down. The partially-cooled skim-milk is now completely chilled by passing from (*d*) over a cooler (*h*), which has ice-water circulating through it. The milk is then pumped by the centrifugal pump (*k*) to a tank for subsequent flavouring treatment.

2. *Holder Process.*—Two main types of apparatus for the "holder" pasteurization of milk are in use: (1) the absolute holders; (2) the continuous or flow system.

The absolute holders retain the milk in one vessel during the thirty minutes or so of pasteurizing treatment. It is usual now to have a series of tanks to form a battery, all insulated by a cork jacket or by a warm-water jacket, into which the previously heated milk flows, and from which the milk is automatically released after a definite interval of time. The advantage of this method over one large container is that greater uniformity of heating is attained, since in the large container a very considerable time may be required to discharge the pasteurized milk, the last portion of milk leaving the vessel being thus heated for a longer time. For margarine manufacture, however, a single vessel is of advantage, since the milk can be heated, pasteurized, cooled, inoculated and ripened all in the one vessel, thus avoiding the contamination likely to be met with in changing from one vessel to another.

The continuous or flow type holders do not retain the milk at rest for a definite period, but simply retard its flow through a heating circuit on its way to the cooler. Of necessity, the flow must be uniform, in order to obtain consistent and trustworthy results. Flow may be induced by gravity or by means of pumps; a feed-cup can efficiently control the former, and careful regulation of valves and pistons the latter. A very convenient type of continuous holder consists of a series of fairly large tubes arranged in successive layers within a heating chamber. The tubes are connected in such a way as to ensure that the milk entering the top tube of the series flows forward and backward through all the tubes, to be discharged from the lower one, the exit pipe taking the form of an inverted U-tube, so that no milk is discharged until all the tubes are full.

Before use, the tubes must necessarily be heated by means of steam or hot water, to avoid the first lot of milk being discharged considerably below the proper temperature. An advantage of this tubular type of pasteurizer is that it admits of easy cleaning, and cleanliness is a *sine quâ non* to success in all machinery dealing with milk for margarine purposes. Pasteurization should be conducted in closed vessels, to avoid the formation of a surface scum, which retards bacterial destruction. Cf. Russell & Hastings, *17th Ann. Rept. Wisconsin Ag. Expt. Station.* Smith, *Journ. Exp. Med.*, **4**. 217 (1899); Ernst, *Milk Hygiene* (Chicago, 1914), pp. 196–7.

Changes in Milk due to Pasteurization

When milk is exposed for a considerable time to temperatures exceeding 63° C. its enzymes are destroyed, a precipitation occurs, to an extent dependent on temperature, of the albumen and soluble phosphates, and there is a viscosity change in the fat globules whereby the "cream line" is affected. The time and temperature effect on milk have been diagrammatically expressed by Dr. North of New York. (See Fig. 4.)

Rupp[1] showed that no chemical change was appreciable in milk heated to 63° C. for thirty minutes. He found that the time required for coagulating milk by rennin is slightly less in milk pasteurized at temperatures up to 65° C. than when unheated; at 75° C. the time is almost doubled. Rupp found also that the acidity is slightly diminished after pasteurizing. On

[1] *U.S. Dept. Agric. Bull.*, **166**, 1–15 (1913).

the other hand, Renshaw and Ware [1] observed an increase in acidity and decrease in alkalinity on pasteurizing milk, and also a rapid decrease in the amount of lactose. However, the concensus of opinion is that the acidity of milk slightly decreases on heating, due to loss of carbon dioxide, and to precipitation of the calcium present.[2]

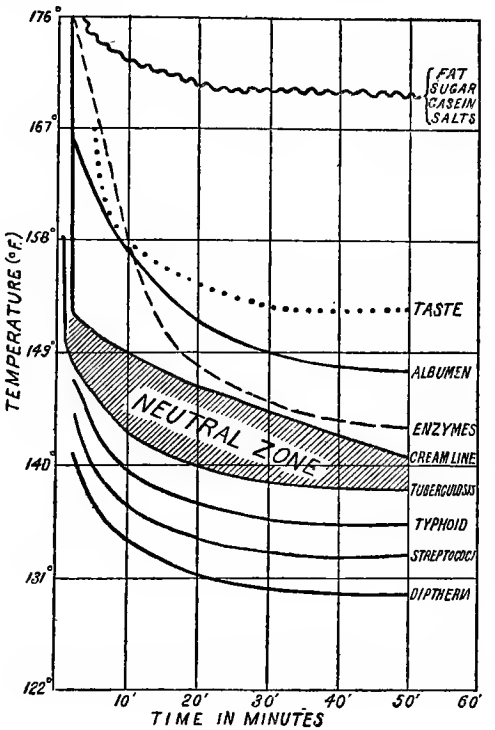

FIG. 4.

Lactose does not undergo caramelization below 100° C., but its loss during the pasteurization of milk is apparently due to certain lactic acid organisms which act rapidly at 80–85° C., but more slowly at 60° C.[3]

From the point of view of nutritional chemistry, it is now

[1] *Journ. Amer. Chem. Soc.*, **32**, 391–6 (1916).

[2] Cf. Jensen and Plattner, *Rev. gén. du Lait*, **4**, 361, 388, 419 (1904–5); Splittgerber, *Zeit. Unters. Nahr. u. Genussm.*, **24**, 493 (1912); Obermaier, *Arch. Hyg.*, **50**, 52 (1904).

[3] Renshaw and Ware, *loc. cit.*

generally agreed that the heating of milk up to even 100° C. for a short period, does not produce changes likely to have any detrimental influence.

It now remains to inquire to what extent pasteurization is efficient in destroying the micro-organisms originally present in the milk, particular attention being devoted to the thermo-stability of the microbe of tuberculosis.

The tubercle bacillus does not multiply in milk, and when present is usually of the bovine type. Whether such tuberculosis is infectious to human adults, has been frequently disputed, but it is now generally agreed that no great danger exists for adults, but that " a considerable percentage of the cases of tuberculosis of children may be traced from the milk supply." [1] The thermal death point of the tubercle bacillus has not been definitely ascertained, but from the numerous investigations made it may be safely asserted that milk maintained at 65° C. for twenty to thirty minutes contains no living tubercle bacilli. (See table.)

Killed at	Authority.	Reference.
60° C. for 10 mins.	Barthel and Stenström.	*Kungl. Landtbruks-Akad. Handl. Tidskr.*, **54**, 649–61 (1915).
65° C. for 15 mins.	De Man.	
68–70° C. for 20–30 mins.	Hewlett and Macfadyen.	Hewlett, *Manual of Bacteriology*, (London, 1914), p. 310.
60° C. for 20 mins.	Mohler.	*U.S. Bur. An. Ind. Report* (1909), p. 187.
60° C. for 20 mins.	Rosenau.	*Hyg. Lab. Washington Bull.*, **42** (1908).
60° C. for 10–20 mins.	Smith.	*Journ. Exp. Medicine*, **4**, 217 (1899).
60° C. for 20 mins.	Schores and Rosenau.	*Journ. Med. Research*, Vol. **26**, No. 1.
60° C. for 20 mins.	Stocking.	*Manual of Milk Products* (New York, 1917), p. 555.
70° C. for 10 mins.	Yersin.	

Mohler, Washburn and Rogers [2] state they have effected the destruction of tubercle bacilli by flash pasteurization at 80° C. Against this must be placed Delépine's [3] work, which has led him to the strong conviction that tubercle bacilli of either human or bovine origin are not always killed even if exposed to 85° C. for fifteen minutes (see also Ernst, *Milk Hygiene*, pp. 196–7).

[1] Marshall, *Microbiology* (London, 1917), p. 403.
[2] *U.S. Bur. An. Ind. Rept.* (1909), p. 179.
[3] Reports to Local Government Board, *Food Reports*, No. **21**, 43 (191

The whole question of the destruction of this organism still awaits a conclusive answer.

Of the other organisms present in milk, it is agreed that heating to 60° C. for twenty minutes destroys all of an infectious type.[1] Ayers and Johnston [2] investigating the thermal death-points of different strains of streptococci in milk found all were destroyed by thirty minutes exposure to 74° C. They also found that the

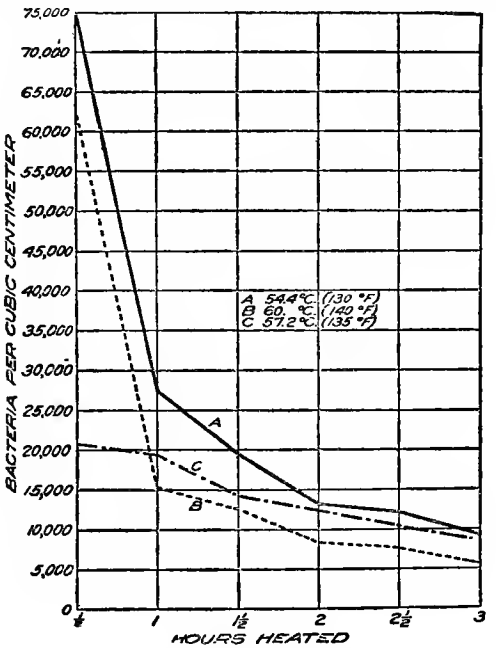

FIG. 5.

(From Ayer's and Johnson's Paper, published by the Department of Agriculture, U.S.A.).

majority of the bacteria in milk are killed during the first thirty minutes heating, even at comparatively low temperatures. The later reduction in numbers is relatively much less rapid. The accompanying graph expresses the bacterial reduction in milk during a three hours' pasteurization of milk at 54·4° C., 57·2° C., and 60° C. respectively [3] (Fig. 5).

When pasteurized milk is obtained for use in margarine manu-

[1] Rosenau. Dept. of Hyg. Washington, *Bulls.*, **41, 42, 57** (1908–9).
[2] *Journ. Agric. Research*, **2**, 321 (1914).
[3] U.S. Bur. An. Ind., *Bull.*, **161** (1913).

facture, the subsequent inoculation with lactic acid bacteria and the rapid curdling and acidification which follow, render the conditions entirely unfavourable for the development of any organisms escaping the pasteurizing process. Even so, the absolute cleanliness and hygienic purity of all the raw milk must be again emphasized, since the after flavour of the finished margarine is practically entirely dependent on the milk treatment before emulsification.

Inoculation and Ripening of the Milk

The cooled pasteurized milk is now ready for inoculation with lactic acid bacteria prior to souring or "ripening." It has long been known that the flavour of butter is to a large extent determined by the type and degree of development of lactic acid organisms in the original cream. The most desirable organism was found to be *B. lactis acidi*, Leichmann, and after suggestions mainly by Storch and Weigmann in 1890, it became common practice to introduce a "pure culture" of such organisms into the cream to induce and control the desired ripening, and thus lead to a good flavour in the subsequent butter. Later such cultures were suggested for use in the making of oleomargarine and renovated butter, and to-day an essential feature of practically all grades of margarine is the aiding of the milk souring by inoculation with pure cultures of *B. lactis acidi*, Leichmann.

These cultures are commercial articles, somewhat akin to yeast, and are termed "starters." Such starters are prepared in the laboratory on the usual bacteriological lines, and sent out in either liquid (milk or beef-broth) or in solid (powder) form. The liquid form tends to undue deterioration of vitality owing to the undesirable by-products resulting from the growth of the culture in a liquid nutrient medium. The powder form is most convenient and keeps well. It is usually obtained by mixing the pure culture with an inert substance, such as lactose, dried milk, chalk, or starch, and dessicating at as low a temperature as possible. The lactic organisms are then in a dormant condition, regaining their activity when introduced into a favourable liquid nutrient medium such as milk.

According to Carrion and Sorel,[1] lactic acid bacilli do not survive drying from a liquid culture even if low temperatures and a vacuum be employed. They found the best results were

[1] *Bull. gén. thérap.*, **163**, 494 (1913).

obtained when the liquid culture was made into a paste with sterilized lactose and chalk, spread into thin layers, and dried at 15° C. *in vacuo*. Lorentz (Eng. Pat. 7898 of 1896) mixes a pure culture of lactic bacteria with a 4% aqueous solution of lactose. To each 75 c.cs. of this mixture he adds 200 grams of potato starch and 50 grams of lactose, and then dries at 30° C. Another method he suggests is to impregnate a mixture of gypsum and lactose in the ratio 3 : 1 with water, press into a cake, and sterilize by heating. The now porous cake is impregnated with a lactic culture, and dried below 30° C. Löloff and Mayer (Ger. Pat. 210988 of 1907) cultivate the desired bacteria in separated milk, and centrifuge, thus obtaining a mixture of casein and bacteria, which, after washing away the milk serum with sterilized water, is compressed and then mixed with sufficient lactose, proteins, or similar inert substance, to yield a dry powder.

In margarine practice, starters are treated as raw materials, and consequently subjected to periodic control tests. The chief points examined are: (1) purity; (2) vitality and fermentative power; (3) capability of ensuring consistent uniform results on repeated propagations; (4) flavour-producing properties.

Purity.—Inoculation from the powder sample is made on to lactose agar and plain agar, carrying out the usual dilution tests, so as to enable any foreign types of micro-organisms to develop. Incubate at 38° C. for forty-eight hours, and then determine the number and nature of the organisms. A starter powder need not necessarily be classed as unfit, if other organisms than lactic acid streptococci be present, for in the growth in pasteurized milk the development of the lactic organisms will be so rapid, and the acidity will also so rapidly increase, that other organisms get crowded out, and a reasonably pure culture in milk will ensue.

Vitality and Fermentative Power.—A good starter should grow rapidly after inoculation into milk, and subsequent incubation between 20° and 30° C. The acidity should then rapidly increase. It is usual in testing these properties to restore the organisms contained in the dry powder to a normal state of vitality by inoculation into sterilized milk, incubating at 25° C. for twenty-four hours, preparing a sub-culture and again incubating for twenty-four hours. The actual quantitative tests are then conducted in varying dilutions in milk, *e. g.* to successive portions of 100 c.cs. of milk, add 0·01 c.c., 0·001 c.c., 0·0001 c.c. of culture. After twenty-four hours at 25–30° C., the condition of the milk is noticed, and its acidity taken. A lactic culture possessing a

high fermentative capacity will curdle milk in twenty-four hours at 30° C. even in a 1 in 1000 dilution. The acidity as estimated by titration against $\frac{N}{10}$ NaOH would average 17 c.cs. for 25 c.cs. of milk.

A quick method for quantitatively determining the vitality of lactic powder starters is described by Bouvet.[1]

Constancy of Properties.—If the powder contains lactic organisms which only slowly produce acid when being cultivated, they lead to conditions favourable to foreign infection, and the flavour and aroma must necessarily deteriorate. For successful work the organisms must be capable of giving consistent results as regards acidity, flavour, and aroma, time after time, as fresh propagations are made. Thus it follows that, apart from the continued use of the starter in works' practice, a laboratory control should also be maintained, and sub-cultures under favourable conditions should be regularly made and examined. Standard conditions of working must be followed in making the propagations, special attention being paid to temperatures and degrees of acidity, since the activity and power of continual transfer of the organisms varies inversely as the acidity of the nutrient medium. For laboratory purposes of retaining over a long period the original activity of *B. acidi lactis*, Leichmann, incubation should be conducted at 18–22° C. in the presence of chalk as an acid neutralizing agent.[2]

Flavour-producing Properties.—To judge a starter for flavour- and aroma-producing properties needs mature experience, and no set rules can be given. Each fresh propagation of a starter can be tested for such properties, and this test may be conveniently included in the preceding one. Sometimes a commercial starter will possess a disagreeable initial flavour, which, however, disappears after two or three propagations.

Inoculation.—For purposes of margarine manufacture the milk is soured by inoculation after pasteurization with suitable quantities of pure cultures, these in turn having been made from a specially-cared-for " mother-starter." The mother-starter is initially prepared from the ferment powder, all of which should be used when once the sealed container has been opened. A package sufficient to make a mother-starter of 3 or 4 quarts contains 37–40 grams of powder. Lactic acid bacteria develop most

[1] *Bull. sci. pharmacolog.*, **20**, 483 (1914).
[2] Makripov, *Zentr. Bakt. Parasitenk. II. Abt.* **37**, 609–22 (1913).

rapidly at 35–42° C., but the most desirable flavour results from incubation at 15·5–24° C. When preparing a culture fresh from a powder ferment, a higher temperature than this is necessary, so that souring is thereby accelerated, with the consequent more rapid production of acid which thus induces greater purity of the culture, owing to the conditions being less favourable for the development of any undesirable organisms present in the powder. In subsequent propagations, as the cultures gain in purity and uniformity, the normal optimum temperature range of 15·5–24° C. can be employed.

To prepare the *mother-starter* pasteurize whole milk at 95° C. for fifteen minutes in an aluminium[1] or heavily-tinned vessel of scrupulous cleanliness. Cool to 33° C. and add the fermenting powder, mixing vigorously. After carefully covering with clean parchment or well-wrung-out boiled cloth, allow to ripen at 30° C., stirring two or three times for the first six hours. In about eighteen hours a soft, custard-like curd indicates that ripening has reached the desired stage, and the vessel is then cooled in ice-water to inhibit further bacterial development. The contents of the vessel must not be stirred or even shaken.

This mother-starter is maintained daily, using exceptional care and cleanliness, since herein lies the basis of the subsequent desirable flavour in the margarine, the milk for which is inoculated from cultures which in turn have been derived from inoculation directly from the mother-starter.

Second Propagation.—For the second day's propagation of the mother-starter, new milk is pasteurized at 95° C. for fifteen minutes, and cooled to 26° C. in cold weather, or to 23° C. if the weather is warm. The cream layer is skimmed off the original mother-starter, and the remaining portion thoroughly stirred. Enough of the culture is then taken out to provide a 1–8% inoculation in the milk now ready. Some workers prefer a 6% inoculation (1 pint starter per 2 gallons of milk), and others a 2·5%. This, however, is entirely a matter of experience, whereby it is known what inoculation percentage is necessary from a given starter to ensure ripening in a subsequent propagation in about sixteen to twenty hours at 18° C. When curdling is complete, the acidity will range from 0·5–0·7% lactic acid, and then it is that the culture contains its maximum number of living organisms. Cool in ice-water, avoiding agitation, and remove the cream layer before use. This is necessary each time, owing to

[1] *Vide* Fillinger, *Zeits. Nahr. Genussm*, **16**, 232–4 (1908).

possible contamination with fungi, especially *Oidium lactis*, which would soon lead to a deterioration in flavour.

For future propagations of the mother-starter, the new milk may be pasteurized at 85° C. for thirty minutes, cooled to about 24° C. in cold weather, or to 19° C. in warm weather, and making a 2·5%, or such other inoculation as to complete the ripening in about sixteen hours when incubated at 18° C. A culture may be successively propagated for long periods, but as soon as any deterioration in aroma and agreeable acid taste is noticed, it should be discarded, and a new mother-starter prepared from a new sample of ferment powder.

Since very large quantities of ripened milk are used in the churning of margarine emulsions, it is necessary to prepare intermediate cultures of considerably greater volume than the mother-starters used. For each 100 gallons of milk to be soured, about 5–7 gallons of skimmed milk are pasteurized at 83° C. for thirty minutes, cooled to 18–24° C. according to atmospheric temperature, inoculated with sufficient mother-starter to ensure ripening in twelve hours at about 18–19° C. This intermediate culture is then added to the main bulk of the milk, contained in suitable vats, after pasteurization by some suitable method, and ripening permitted until the acidity and curdling are at that stage suitable for permitting the milk to be emulsified with the necessary oils and fats to give a desirably flavoured margarine.

The Ripening Process.—Grimm [1] has shown that lactic acid fermentation with pure cultures of *B. acidi lactis*, particularly at 35° C., can be divided into four main phases—

(*a*) A period of habituation lasting about four and a half hours, wherein there is a marked increase in the number of organisms, and no acid produced.

(*b*) A second period lasting about twelve hours, wherein there is exhibited increasing vitality of the bacteria, the optimum being attained about fourteen hours from the beginning of the first phase.

(*c*) A phase of decreasing vitality or acid-producing power. This phase lasts about sixteen hours.

(*d*) The bacteria finally lose their capacity of producing lactic acid.

For sub-cultures or repeated propagations for margarine purposes, it is essential to re-inoculate during the second phase of

[1] *Zentr. Bakt. Parasitenk. II. Abt.* **32**, 65–70 (1911).

marked vitality. In this connection attention may be drawn to a proposed method by Northrup [1] for conserving the vitality of lactic bacteria for long periods without frequent transfers. He found that certain acid-reducing yeasts and lactic bacteria exhibit the phenomenon of associative action in mixed culture in milk or in whey, a condition of equilibrium being attained, whilst the lactic bacteria retain their normal activity for many months.

It might quite reasonably be thought that in souring or ripening milk by means of pure cultures of *B. acidi lactis*, a certain acidity or definite hydrogen ion concentration would be reached at which the characteristic flavour indicative of the fully-ripened milk would be first noticeable. It has been shown, however, that no such definite relation exists, and the compound (or compounds) responsible for the flavour in question, which can be detected by the smell alone, or by the combined senses of taste and smell, is in all probability the product of bacterial action.[2] Milk to which lactic acid is added does not furnish the flavour characteristic of milk naturally soured. The flavour of soured milk is quite apparent before the milk begins to taste " acid."

The casein of milk begins to coagulate when the hydrogen ion concentration ranges from $2{\cdot}3 \times 10^{-5}$–$1{\cdot}7 \times 10^{-5}$ (van Slyke and Baker); Allemann,[3] precipitating casein from milk by dilute mineral acids, found the optimum hydrogen ion concentration = $2{\cdot}5 \times 10^{-5}$, and the minimum $1{\cdot}3 \times 10^{-5}$. Thus it is the actual hydrogen ion concentration (C_H) and not the nature of the acidity, that matters. The total acidity by titration varies from 43–80 c.cs. of 0·1N acid per 100 c.cs. of milk, and during the period of actual coagulation of the casein the hydrogen ion concentration remains constant, whilst there is a slight increase in acidity as measured by titration.

Coagulation completed (from thirty to sixty minutes at 25° C.) the acidity again develops, and in fully soured milk is usually between 90 and 100 c.cs. expressed as 0·1N acid.[4]

It is interesting to note here that inoculation of milk with *Streptococcus lacticus* only slowly develops acidity, and with *Bacillus bulgaricus* rapidly induces acidity which may finally reach a figure equivalent to over 200 c.cs. of 0·1N acid per 100 c.cs. milk (at 25° C.).[5]

[1] Mich. Agric. Expt. Station, *Tech. Bull.*, **15**, 3–35 (1913).
[2] Van Slyke and Baker, *Journ. Biol. Chem.*, **35**, 147–78 (1918).
[3] *Biochem. Zeit.*, **45**, 346 (1912).
[4] *Journ. Biol. Chem.*, **35**, 172 (1918).
[5] Van Slyke and Baker, *loc. cit.*

CHAPTER VII

THE MANUFACTURE OF MARGARINE (*continued*)

THE preparation of the fatty portion of the margarine being completed, and the milk suitably ripened or soured, it is now necessary to mix the two together as intimately as possible. An emulsion is produced, and it is absolutely essential to make as perfect an emulsion as possible. This stage of the manufacturing process is of first importance. The object of emulsification is to imitate the emulsion found in cream and milk, where the fat globules have diameters ranging from 0·01 mm. to 0·0016 mm. and remain as discrete particles. The general theory involved in this operation is worthy of study, and is dealt with at length in the next chapter.

Until recently most margarine factories depended on the "margarine churn" for making the emulsion. This churn (illustrated in Fig. 6) is jacketed, allowing of temperature regulation by either introducing hot or cold water to the jacket. Milk is admitted to the churn, which is provided with suitable agitating devices, *e. g.* rapidly-revolving baffle plates, and when at the desired temperature, the liquid fatty constituents are slowly run in, constant agitation being maintained. It is essential to pour the oils into the milk, not *vice versa*.[1] For a ton of the melted oils and fats, various quantities of milk may be used, depending on market conditions and the quality of margarine being made. A volume of 30 gallons of milk with 30 gallons of water is convenient, though the emulsion may be made in such proportions as to yield a final product containing about 16 % of water. This is a matter largely depending on the subsequent treatment of the emulsion.

When emulsification is complete the temperature is reduced to between 25° C. and 35° C., depending on the composition of the fatty portion, and the custard-like emulsion is run off for cooling. The object of cooling is to "fix" the equilibrium

[1] Blichfeldt, Eng. Pat. 4505 (1912); *vide* also chap. viii, p. 71.

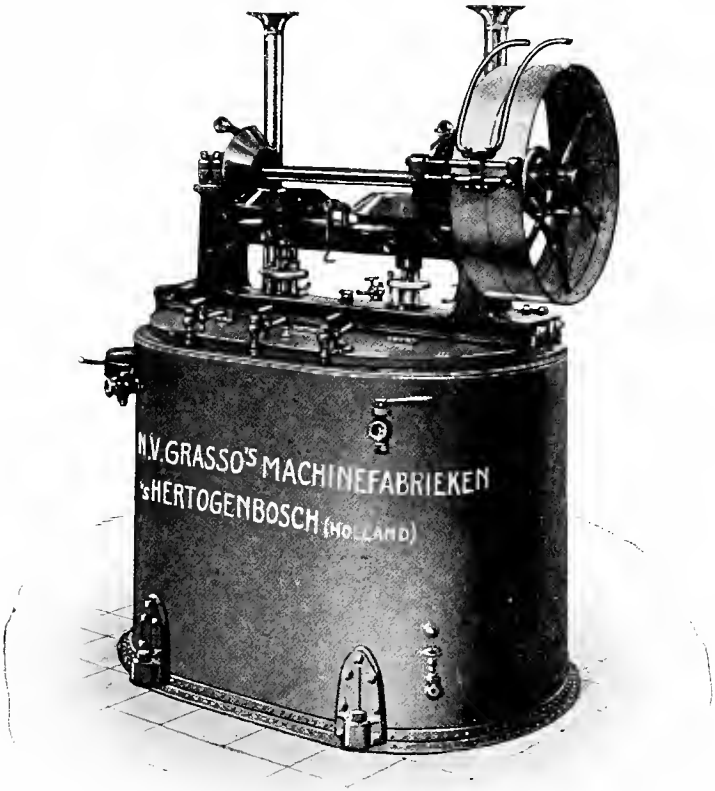

Fig. 6. BULK CHURN.

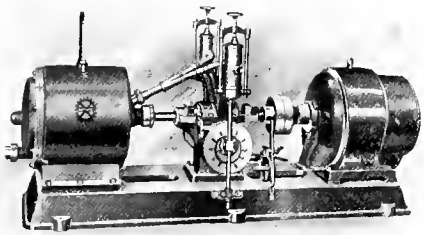

Fig. 7. ELECTRIC CONTINUOUS EMULSIFIER.

condition attained in the emulsion, whereby the desirable intimate and finely-divided mixture is maintained in the solid state. Any "breaking" of the emulsion would lead to the formation of unemulsified fat, whereby the texture of the final product would be harmed. A margarine possessing a smooth, butter-like texture can only result provided a perfect emulsion is attained, and this subsequently cooled as rapidly as possible.

Although the old type of margarine churn leads to excellent results, it occupies much space and absorbs considerable power for driving. Consequently several improved emulsifying machines have been devised, aiming at the *continuous* production of an emulsion. This is a great advantage, since the machines occupy much less space, and do not need refilling in 1 or 2 ton batches, as did the older or bulk type of churn. The principle underlying continuous churns is the simultaneous admission of the oils and the milk into a chamber, preferably thermo-regulated, where some suitable device ensures intimate mixing or emulsification.

Schröder [1] was one of the pioneers in this type of machinery, and his English patent of 1905 describes a continuous churn which served to homogenize the emulsion. Risberg [2] also proposed a homogenizing apparatus comprising a centrifugal drum provided with wings. The oils and milk are led into the drum separately, and this revolving at 6000 to 8000 revolutions per minute, intimately mixes the materials owing to the action of the wings. The mixture is caught by a stripping-off pipe containing fine holes, which causes the emulsion to leave by this stationary exit in a very finely divided condition.

A method involving the passage of the oils and milk between a stationary grinding member and a continuously rotating friction disc pressed against this, gives a good emulsion. Patents introducing such a principle have been granted to the Altonaer Margarine-Werke Mohr & Co.,[3] and to Davis.[4]

Another type of continuous churn widely adopted in modern margarine factories is that patented by Silkeborg Maskinfabrik Zeuthen and Larsen in 1914.[5] This machine consists of a horizontal cylinder provided with a jacket for thermo-regulation, and carrying a shaft fitted with discs or blades. Baffle plates are situated (stationary) in the cylinder, containing small holes,

[1] Ger. Pat. 309717 (1917); Eng. Pat. 25404 (1905).
[2] Eng. Pat. 25890 (1907).
[3] Eng. Pat. 1227 (1910).
[4] U.S. Pat. 1269399 (1981).
[5] Eng. Pat. 4657 (1914).

the diameters of which decrease the nearer the baffle plate is to the exit pipe. Oils and milk are led in by separate pipes through alterable pumps (whereby volume relations can be maintained), and the rapidly-rotating stirrers (1500 r.p.m.) intimately mix the ingredients and force the mixture through the small holes in the baffle plates. By churning at 25–35° C. a very good emulsion is formed, the maximum output of the machine being as a rule 3 tons of margarine per hour. A 16-horse-power electric motor may be conveniently coupled to form a direct drive, when an average ampèrage of nineteen ampères will be read. Fig. 7 shows a typical Silkeborg machine.

Blichfeldt [1] has devised a continuous emulsifier provided with a rotating disc pierced with slots, which is carried on a hollow shaft in a chamber whose walls are very close to the casing surfaces. The milk is introduced through the hollow shaft, and the fatty constituents are led through openings in a special tube passing across the chamber and communicating with the interior of the casing through various small passages at different distances from the axis. A similar idea is embodied in an emulsifier due to Leitch,[2] and to Christensen.[3]

Chilling the Emulsion.—After the preparation of a satisfactory emulsion, cooling or chilling is necessary, and this must be done expeditiously. Two systems are in use: the wet method and the dry method. The former is the older method, in which the emulsion is solidified by suitable contact with ice-cold water. This has the disadvantage of washing out some of the milk and its flavouring constituents, and of exposing the product to bacterial infection. Only water of a sound hygienic quality should be employed.

The earliest method was to discharge the emulsion into a tank of cold water,[4] whence the solid "crystals" of margarine could be skimmed off. Later, the emulsion was discharged on to a slanting chute and met by a sheet of ice-cold water,[5] an idea which soon led to the introduction of the ice-water spray, still used in several factories in this country.[6] On this system the emulsion falls on to a chute, where it is instantly met by a spray of the cooled water under a slight head. Solidification at once takes place, and the small globules or "crystals" of margarine float on the excess of water like yellow snow, and fall into a

[1] Eng. Pat. 18048 (1914).
[2] U.S. Pat. 1266501 (1918).
[3] Eng. Pat. 129757 (1919).
[4] Brewer, Eng. Pat. 1547 (1881).
[5] Coad, Eng. Pat. 20438 (1896).
[6] Hargrave, Eng. Pat. 22873 (1896).

trough beneath, where drainage is effected. Such "crystals" usually contain from 30–35% of moisture, irrespective of the composition of the fatty portion of the emulsion. Fig. 8 indicates the general arrangement of the plant for making margarine on this system. An improvement of this method has been made by Larsen and Zeuthen,[1] who pass the water and emulsion by worm conveyors in opposite directions. They claim that less water and less cold are required, an important consideration in view of the fact that for each ton of margarine made on the

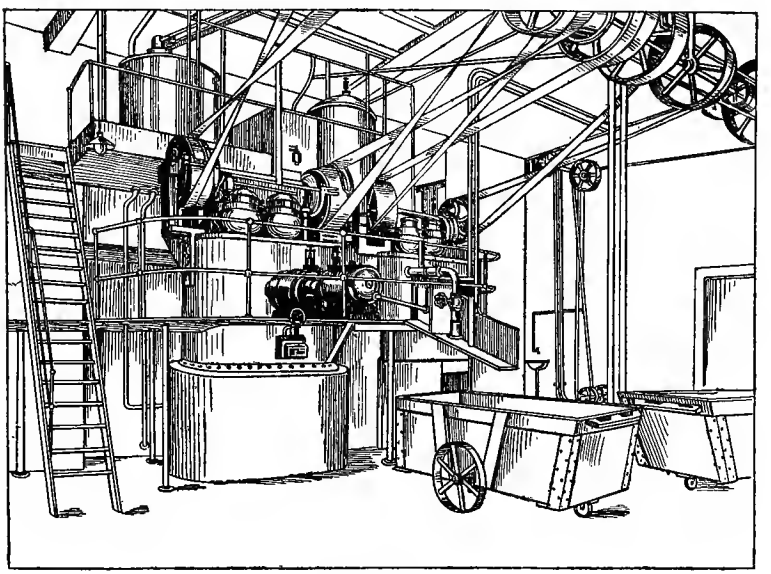

FIG. 8.

ice-water spray system, from 2000 to 2500 gallons of water at 33–35° F. are expended.

The more modern factories have dispensed with the wet method of chilling, and employ some other cooling device, characterized by the fact that a more rapid and more equal cooling is effected out of contact with the cooling medium.

The most notable apparatus is the cooling-drum invented by Schöu,[2] and illustrated in Fig. 9. The mechanism comprises "a multiple cylinder apparatus, cylinders of which rotate in

[1] Eng. Pat. 110872 (1917). [2] *Ibid.*, 12561 (1907) and 1160 (1909).

opposite directions and are adjustable towards each other, the arrangement being such that the thickness of the spread-out layer or film of emulsion is determined by the smallest distance of the cylinders from each other." Cooling is effected by means of brine at about $-7°$ C., and this is circulated through the revolving drums. The emulsion falls on to the drums and is automatically fed so that a thin uniform layer only is applied, which instantly solidifies and is stripped off as flakes by means of adjustable knives. The apparatus is arranged so that the fine flakes have a temperature of about 8–10° C. when stripped off.[1] (Fig. 10 shows an American cooling drum.)

The dry method yields most satisfactory margarines and is far more hygienic and economical than the wet methods. Several other types of cooling drums have been devised: Rasmussen[2] combined the revolving drum with the wet method, whereby the emulsion was fed on to a revolving cylinder immersed about two-thirds of its diameter in water; the cooled product is removed by scrapers. This method suffers from the usual disadvantages of any system cooling the emulsion in actual contact with the cold medium. Christensen and Lauridsen[3] claim a revolving drum on to which the emulsion is fed so as to yield a film of any desired thickness. A movable bar regulates this thickness, and being warm, smooths off the hardened film. Fixed scrapers strip off the product as flakes.

A. Jurgens' patent of 1914[4] describes a type of cooling drum which is now meeting with increasing favour. A revolving cylinder maintained (by circulating warm water) at the temperature of the emulsion to be treated dips into the latter, and transfers the emulsion to another revolving cylinder (brine cooled) of about eight to ten times the diameter of the first. The two cylinders revolve in the same direction, and the thickness of the emulsion film can be regulated by altering the speed of the feeding cylinder. The quicker the feeding surface moves relatively to the cooling surface, the thicker will be the layer transferred. The fat is retained for almost a complete revolution of the cold drum before being removed by a scraper. (See Fig. 11.)

Instead of using ice-water or brine-cooled drums as the cooling agent, several proposals have been made to use cold air.[5] Jamie-

[1] Eng. Pats. 4278 (1913) and 17616 (1913).
[2] Eng. Pat. 29831 (1910).
[3] Eng. Pat. 20568 (1912).
[4] Eng. Pat. 10863 (1914).
[5] *Vide* Scheffel, Ger. Pat. 116755 (1899).

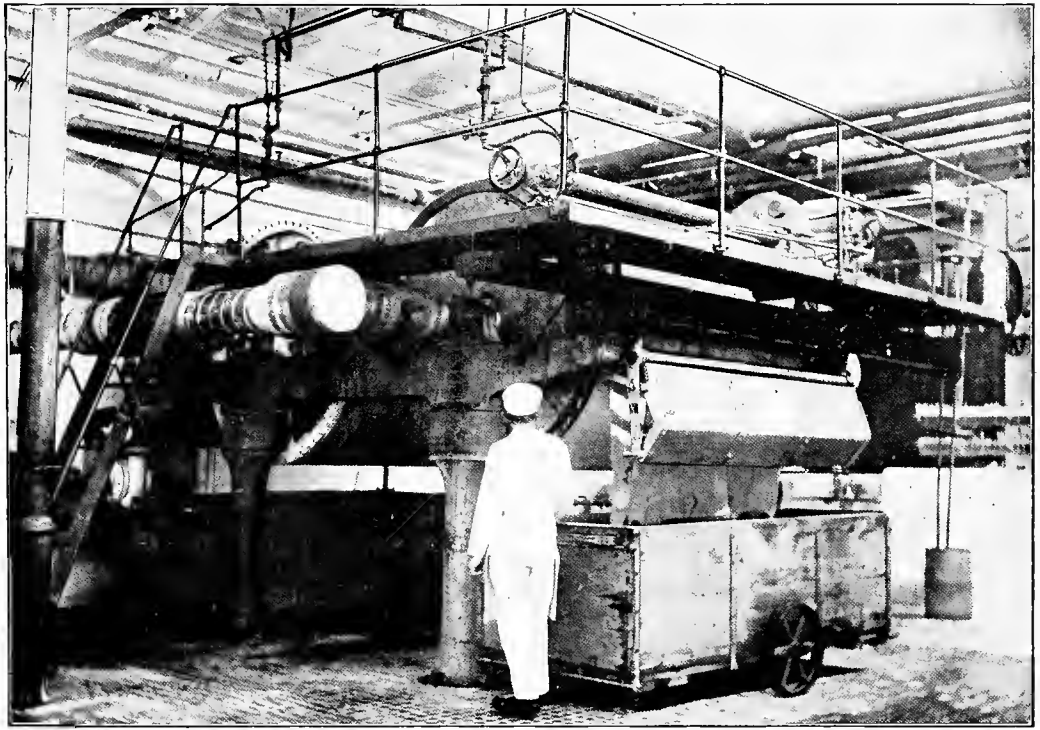

Fig. 9. SCHOU COOLING DRUMS.

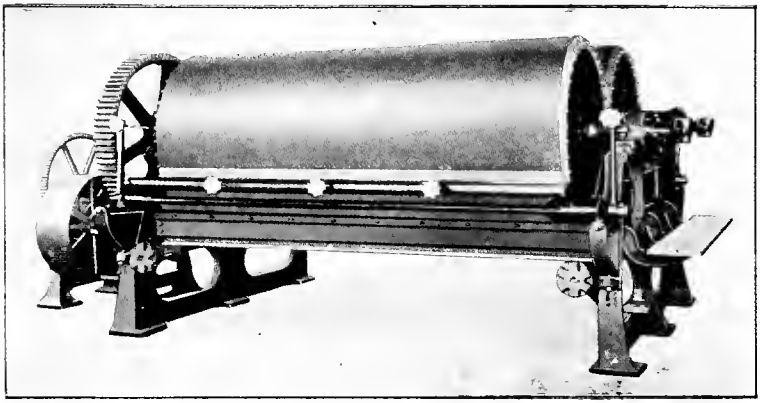

Fig. 10. AMERICAN COOLING DRUM.

(The Allbright-Nell Co., Chicago.)

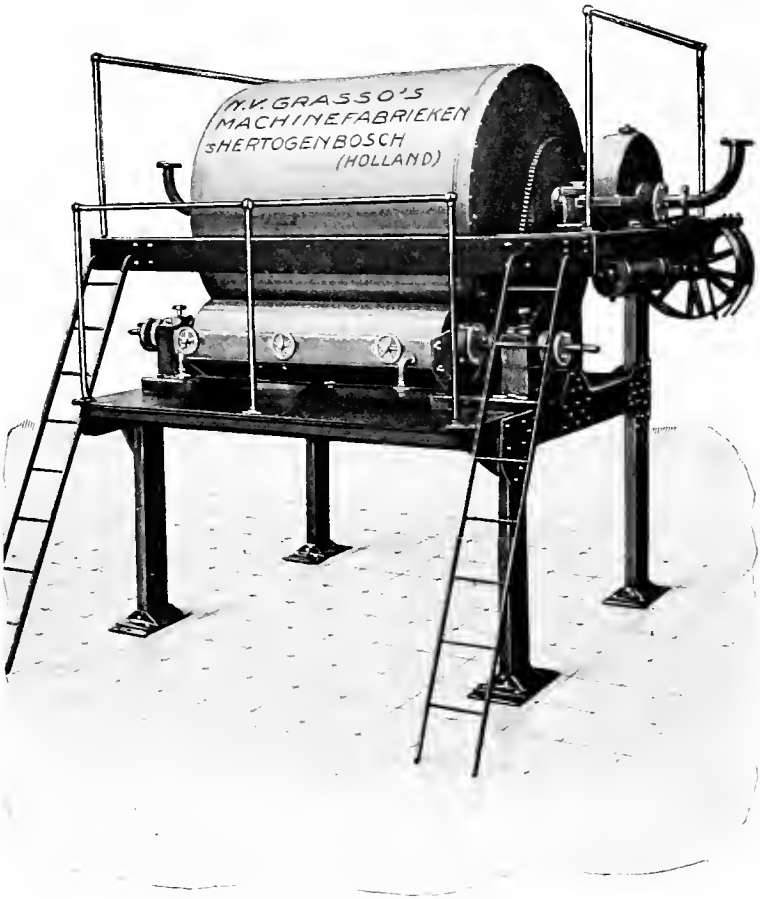

Fig. 11. JURGENS COOLING DRUM.

son [1] patented a method in which the margarine emulsion is fed on to a travelling band running through a cold-air chamber. A scraper is arranged in connection with this carrier, to remove the margarine after it has been cooled. In this connection attention is directed to a patent by Blichfeldt,[2] for avoiding the contamination of the margarine at all stages of the manufacture from impure air.

A novel method of solidifying a margarine emulsion, and one which has much to recommend it on purely physico-chemical grounds, apart from economic considerations, is due to Erslev.[3] His idea is to atomize the emulsion as a mist by means of spraying nozzles within a chamber containing cold air or other indifferent cold gas. The emulsion passes through the nozzles under a very high pressure up to several hundred atmospheres, whilst the gaseous cooling agent is passed in counter current by means of a ventilating device, drawn off, cooled again, and returned to the process. The cooled fat falls to the floor of the chamber and is carried away by a travelling belt or worm conveyor. A later modification [4] introduces a vacuum chamber in which is suspended a rapidly rotated disc. The emulsion is fed on to the disc, flung off as a fine spray, and by evaporation of the moisture cooled to a solid powder. A process quite the reverse of this, in which the emulsion is sprayed into a hot-air chamber for concentration, has been proposed by Dick.[5] Erslev claims that his product has a butter-like consistency and keeps well.

In all these methods the cooled emulsion, whether in the form of "crystals," flakes, or powder, requires further treatment to consolidate the mass so as to resemble butter. Such treatment demands rolling or kneading the mass in revolving drums fitted with rollers, or on tables specially adapted to "work" the mass to the required consistency. Also in certain instances, notably where the wet method of chilling is employed, intensified rolling is necessary in order to reduce the water content of the margarine to within the legal limit of 16%. It is usual to warm the "crystals," flakes, or powder before subjecting to such kneading action, so as to assist cohesion of the particles. Such temperating may take place by allowing the cold product to remain in a warm room (about 20–23° C.) for several hours, or the heating may be expedited by means of hot air or steam.[6]

[1] Eng. Pat. 20292 (1911).
[2] Eng. Pat. 4508 (1912).
[3] Ger. Pat. 289262 (1913); cf. also Grelck, U.S. Pat. 1144539 (1915).
[4] Eng. Pat. 134815 (1918).
[5] U.S. Pat. 1258996 (1918).
[6] Eng. Pat. 4278 (1913), Schöu.

The accompanying plates illustrate some common types of kneading machinery for margarine. Fig. 12 represents a rolling-mill, only found in a few factories now; Fig. 13 depicts a revolving churn or drum fitted with two sets of rollers which serve to knead the mass together; Fig. 14 represents a type of table fitted with a fluted roller. The margarine is fed on to the table, which is then caused to revolve, and the mass passes beneath the roller, which is also in motion. Revolving drums constitute the most efficient and usual machinery employed.

The rolled product is now mixed with salt, preservative, and any flavouring substance desired. This mixture is accomplished in a blending apparatus, of which Fig. 15 is a common type.

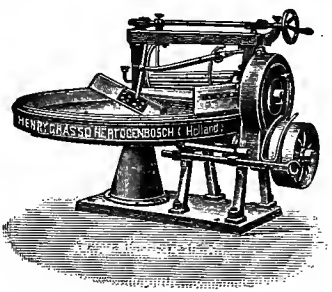

FIG. 14.

The margarine is then ready for packing in boxes, tins or kegs, or in small pats of any desired shape and weight.

Numerous patents have been obtained for the addition of chemicals to margarine, either to improve the texture, or, more often, to impart a more butter-like flavour. The more important of these blender additions include: glucose,[1] lecithin (0·1–2%),[2] refined spirit (7–9%),[3] saccharine,[4] glycerine,[5] "emulsin" or "synaptase" from nuts,[6] glycerides,[7] egg-yolk preparations,[8] animal or vegetable wax (0·5–5%),[9] albumen from cereals such as wheat,[10] egg-albumen,[11] fruit acids such as citric acid,[12] ozonized air,[13] cholesterol and its esters,[14] etc.

[1] Eng. Pats. 3867 (1880); 27487 (1911), Schmitt.
[2] Eng. Pat. 46 (1905), Jurgens.
[3] Eng. Pat. 18499 (1907), Kolesch.
[4] Eng. Pat. 19158 (1889), Heinz.
[5] Eng. Pat. 21103 (1895), Gray.
[6] Eng. Pat. 12355 (1898), Liebreich.
[7] Eng. Pat. 15535 (1898), Wohlgemuth.
[8] Eng. Pats. 4711 (1899); 21626 (1900), Neisse and Boll.
[9] Eng. Pat. 22905 (1900) Pellerin.
[10] Eng. Pat. 8099 (1903), Jurgens.
[11] Eng. Pats. 27449 (1913), Cronholm; and 14872 (1913), Schmitt.
[12] Eng. Pat. 7296 (1913), Kauffmann.
[13] Norwegian Pat. 19107 (1909), Svendsen.
[14] Eng. Pat. 7620 (1901), Sprinz.

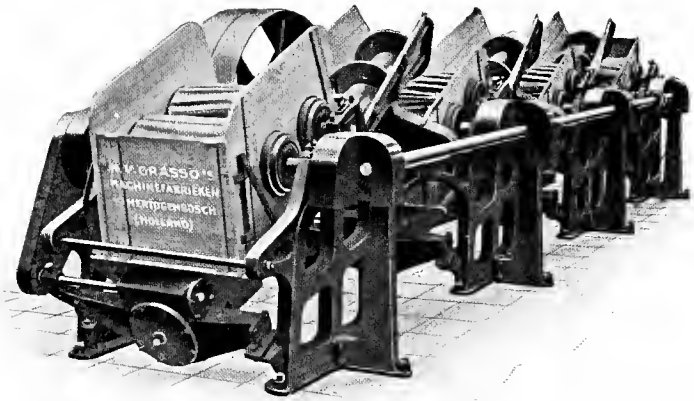

Fig. 12. MARGARINE ROLLING MILL.

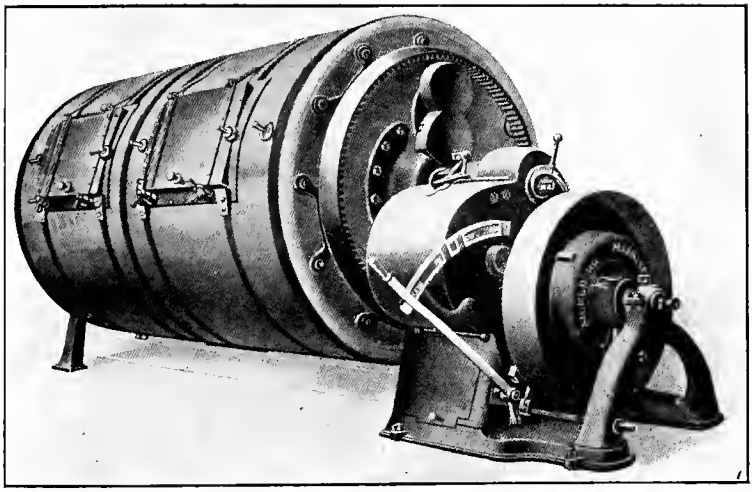

Fig. 13. MARGARINE WORKING CHURN.

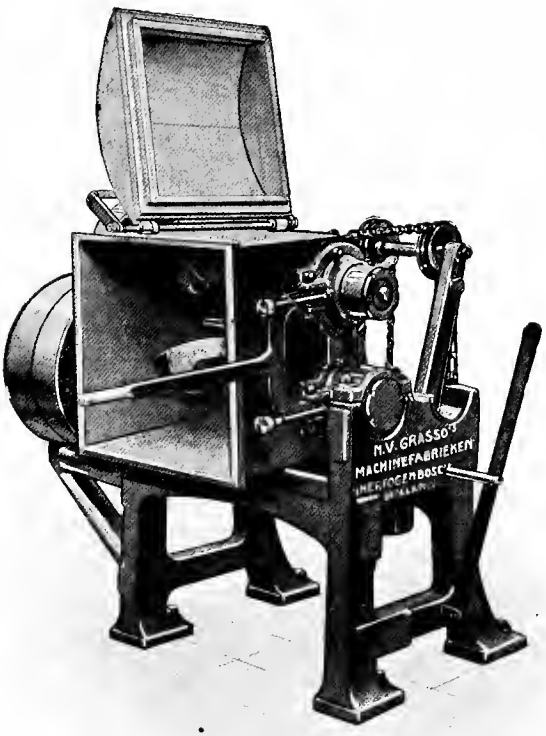

Fig. 15. BLENDER MACHINE.

Some of these additions are useful in serving to make a margarine cook better, *i. e.* froth and brown on heating. Addition of dried milk, casein, albumen, or egg-yolk induces such an effect. The tentative flavouring devices find extremely little favour, if any, and are to be deprecated. The truly desirable flavour of butter will be best secured by working with sound raw materials, good milk soured with a carefully-chosen culture of lactic acid bacteria, and a strict observance of hygienic conditions throughout. Future research will undoubtedly lead to the discovery of a synthetic butter flavour, or to a method of bacteriological control which will produce the desired flavour.

With regard to suggested treatment of the milk likely to produce a good flavour in the margarine, the following English patents should be consulted: Borgström (6394 of 1907), Buer (18201 of 1909), Blichfeldt (4504 of 1912), Mann (15311 of 1905), Mott (3138 of 1913), Poppe (18500 of 1898), Revis (121033 of 1918), Rydberg (116603 of 1918), Wilson (19535 and 24050, both 1911), Witte (1744 of 1910, and 10972 of 1912).

CHAPTER VIII

THE THEORY OF EMULSIFICATION

EFFICIENT emulsification, leading to the formation of an emulsion as fine and as stable as possible, is absolutely essential in order to make a margarine of a butter-like texture and consistency. Within recent years our conception of emulsions has radically altered, and the rules governing their formation have been elucidated to a considerable extent. But practically no work has been published on *solid* emulsions, and many problems in colloid chemistry need to be studied in the case of solid emulsions of which butter and margarine are typical examples.

A margarine emulsion is far from being a simple case of emulsions in general, and any attempt to outline its physical chemistry must necessarily be based on first principles.

An emulsion is a heterogeneous system in which a liquid is dispersed as droplets in some other liquid with which it is only partially, or not at all, miscible. Theoretically two types of emulsion become possible according to which liquid is dispersed as drops. The two phases are distinguished as the "disperse phase" and the "continuous medium," the former appearing as globules dispersed in the latter. The outstanding characteristic of such a system is the relatively large interfacial separating layer between the two phases, whereby surface phenomena become pronounced. The expression "interfacial separating layer" refers to a very attenuated layer due to the interpenetration of both phases. This layer is only a few millionths of a centimetre thick. For noticeable effects to come into play, the specific surface, *i. e.* surface volume, requires to be increased to at least 10^4 times the initial value. Limiting this discussion to a consideration of oil dispersed in water, it is found that there is an interfacial tension between the oil and the water, since the surface energy has increased, and hence the total surface of the oil phase tends to diminish. It has been demonstrated that if a third substance be added, which will affect the value of this

interfacial tension, concentration phenomena appear, *i. e.* the concentration of the added substance will be different in the main bulk of the continuous phase from what it is at the interfacial separating layer.

Willard Gibbs[1] worked out the mathematical relations involved, his simplest equation appearing in the form—

$$U = -\frac{C}{RT} \cdot \frac{ds}{dC}.$$

where U is excess concentration at the boundary surface in grms./cms. and C the concentration of the solute in bulk of solution.

Since ds/dC is the differential coefficient of the function connecting surface tension and concentration, it follows that it is positive when the solute increases the surface tension, and negative when it decreases it. Consequently U is *positive* when the added substance *lowers* the surface tension. That is to say, the added substance will be adsorbed in the boundary surface between the oil particles and water, if by its adsorption the interfacial tension is reduced; this is a most important result, and essential to the theory of emulsification in general.

Instead of Gibb's formula, a purely empirical relation due to Freundlich[2] is sometimes employed in investigations on emulsions and adsorption. It is: $x/m = aC^{\frac{1}{n}}$, where x is the amount of substance adsorbed, m the mass of the adsorbent in grams, C the equilibrium concentration when adsorption is effected, and a and n are constants, n being greater than unity. The equation is that of a general parabola, and may be written in a simpler logarithmic form, $\log x - \log m = \frac{\log C}{n} + \log a$. This is the equation of a straight line, as may be seen by plotting $\log x$ against $\log C$, the two variables.

It is very striking that at low concentrations the adsorbed quantities are relatively great,[3] and only increase slowly with rise in concentration. Adsorption is reversible, *i. e.* there is an equilibrium state, which as a rule is very quickly attained. In most cases rise of temperature favours adsorption at liquid interfaces.

In dealing with emulsions, and taking only the very simple

[1] *Trans. Connecticut Academy*, Vol. III, 439.
[2] *Zeits. phys. Chem.*, **57**, 392 (1907).
[3] Milner, *Phil. Mag.*, **13**, 96 (1907)

case of pure oil and water, it is theoretically conceivable to prepare two sets of emulsions over a whole range of concentrations, one set to consist of oil dispersed in a continuous medium of water, the other set of emulsions to be water dispersed as drops in an oil medium. It is only recently that the latter case has been really investigated, particularly by American chemists. With either set of emulsions no inhibiting upper limit exists for the ratio : volume of disperse phase to volume of continuous medium.

Oil-in-water emulsions may be made in several ways, the chief of which is agitation, or beating up of the oil in water, or aqueous solution. Owing to the large extension of surface area of the oil, a large amount of surface energy is produced. If this energy can be reduced, the process of emulsification becomes much easier; such a reduction in surface energy may be attained by using an aqueous solution of low-surface tension, *e. g.* solutions of soap or gelatin. This third substance is then termed an emulsifier or an emulsifying agent.

The oil becomes dispersed as drops of small diameter, the smallness of the drops depending on the efficiency of the emulsification apparatus employed. Other things being equal, the smaller the drops the more stable or permanent will be the emulsion. In cream, for instance, the butter-fat globules range from 0·003–0·005 mm. diameter, and in milk from 0·01–0·0016 mm. In general we may say that 10^{-5} cms. is the usual order of the diameter of emulsion globules. These drops are electrically charged, this charge bearing a negative sign, since the oil phase has a lower dielectric constant than the water phase. Between the oil globules and the continuous medium there exists a potential difference, amounting to about 0·05 volt. Ellis[1] showed this P.D. to be independent of the kind of oil used or even the degree of purity of any given oil. This contact potential or P.D. is very intimately connected with the stability of an emulsion, such stability decreasing as the P.D. is made to decrease, *e. g.* by adding mineral acid. Until recently it was assumed that when the P.D. decreased to zero the stability became *nil*, *i e.* a complete breakdown of the emulsion occurred. Powis,[2] however, showed that there was a critical potential, viz. : $\pm$0·030 volt, at which an emulsion is most stable. If the P.D. be now made to decrease, coagulation follows with a velocity which is approximately the same for all values of the potential, and the point of complete

[1] *Zeits. Phys. Chem.*, **78**, 321–52 (1911).
[2] *Ibid.*, **89**, 186–212 (1914).

breakdown of the emulsion need not necessarily coincide with a zero potential, the so-called iso-electric point.

Finally, another property possessed by the oil globules in an emulsion is that of Brownian movement, which helps to keep them in a state of suspension. This motion is due to the bombardment of the oil particles by the molecules of the continuous medium, and this bombardment being a series of unco-ordinated collisions, the oil particles execute a zig-zag motion, which varies inversely as the size of the globules. When their diameter exceeds about 4μ, *i. e.* 4×10^{-3} mm., the Brownian movement ceases.

If an attempt is made to emulsify a pure oil in distilled water, it soon becomes evident than an extremely weak and unstable emulsion results. To obtain an emulsion of even moderate concentration, a third substance must be added. Thus Pickering[1] by using soap made a very stable emulsion of 99% oil in 1% water. By means of a suitable third agent, the reverse type of emulsion is possible, viz.: a concentrated emulsion of water in oil. Thus Schlaepfer[2] made an emulsion of 70% water in 30% kerosene, using soot as an emulsifier. Moore[3] has obtained similar results using lamp-black.

Many theories have been advanced to explain the process of emulsification, and to account for the stability and permanence of the subsequent emulsions, but until recently they only dealt with the special case of the oil-in-water type of emulsion. A useful theory must be perfectly applicable to both types—

(A) Ostwald[4] believed the chief cause determining the *type* of emulsion was the relative concentrations of the two phases. According to his views, as the concentration of one phase increased a critical point was reached beyond which the continuous medium then became the disperse phase. Starting now with an emulsion of the new type, and increasing the concentration of the disperse phase, a new critical point was reached which, however, did not coincide with the first.

Ostwald assumed two things: (1) that the emulsion particles were spheres of equal size, and (2) that the continuous medium changes to drops when the original globules come into contact. The critical volume-ratio of the two phases would then be approximately 74/26. But since an emulsion consists of *liquid* phases

[1] *Journ. Chem. Soc.*, **91**, 2002 (1907).
[2] *Ibid.*, **113**, 522–6 (1918).
[3] *Journ. Amer. Chem. Soc.*, **41**, 940–6 (1919).
[4] *Kolloid Zeits.*, **6**, 103 (1910), and **7**, 64 (1910).

at ordinary temperatures, deformation of particles may occur, and coalescence of the globules does not necessarily follow their coming into actual contact. A certain elasticity of shape may be conceived whereby the globules can be "squashed down until the space between them is only vanishingly small." [1] Ostwald's theory is all the more difficult to accept in view of the fact that as early as 1891 Pickering had made his well-known experiments on the emulsification of even 99% oil in 1% water. Still another objection to the theory is contained in the fact that in all emulsions the globules are by no means equal in size.

Briggs and Schmidt [2] showed in their work on the emulsification of water in benzene that, given the right emulsifying agent, the relative concentrations of the water and benzene were quite immaterial.

(B) Another theory advanced to account for emulsification was the viscosity theory, according to which an emulsifier had to be a viscous substance, or lead to a viscous continuous medium when in solution. Undoubtedly high viscosity favours emulsification, but many instances are known where the viscosity idea utterly fails. Thus Hillyer [3] has pointed out that 50% glycerin and 6% gum solutions of marked viscosity will not emulsify kerosene or even so viscous an oil as cottonseed oil, whilst dilute soap solutions with only a small viscosity are excellent emulsifying agents, and will easily emulsify kerosene and cottonseed oil.

(C) Pickering [4] put forward an interesting theory of emulsification in which the main idea is that films of insoluble particles envelop the oil globules. He showed that the insoluble basic salts of iron, copper, nickel, and aluminium are good emulsifying agents with respect to mineral oil and water. He showed, too, that clays, lime, silica, plaster of Paris, and other insoluble powders could act just as well. In this connection it is interesting to recall Schlaepfer's very stable emulsions of water in kerosene, where the emulsifying agent was also an insoluble solid, viz. :— soot. Pickering expressed the idea that emulsification depended solely on the size of the particles constituting the precipitate, and that the average size of the globules in an emulsion was dependent on the size of the particles of the emulsifier employed.

We now know that in the use of insoluble emulsifiers the main question to be asked is, will the solid be more readily wetted by

[1] *Journ. Phys. Chem.*, **16**, 179 (1912).
[2] *Ibid.*, **19**, 478–99 (1915).
[3] *Journ. Amer. Chem. Soc.*, **25**, 513 (1903).
[4] *Journ. Chem. Soc.*, **91**, 2002 (1907).

the oil or by the water? That phase which wets the solid the more easily will become the continuous medium, and the other phase will be dispersed in it. The ease of wetting of a solid by a liquid is a function of the interfacial tension between the two phases, and the lower this tension, the more readily does wetting take place. Hence, if the solid particles of an emulsifying agent have a lower interfacial tension against water than against oil, it follows that the resultant emulsion will be of the oil-in-water type, and *vice versa*. The film or pellicle so described by Pickering has been investigated by Clowes,[1] who actually visualized several cases. The particles constituting the film must necessarily possess little power of agglomerating, since their main effect is to coat or envelop the oil globules in such a manner as to prevent these coalescing when brought into close contact.

An extension of this theory has been applied to liquid emulsifiers, such as solutions of soaps, gums, gelatin, etc. By adsorption at the interfacial separating surface of the oil-drops there is assumed to be present a solid film derived from the emulsifying agent present in solution in the continuous medium. The idea of a surface film or pellicle surrounding emulsified particles must necessarily postulate an equilibrium condition, *i. e.* particles of the solute are continually being re-dissolved and re-precipitated. Cases of spontaneous de-emulsification, *i. e.* separation of the oil and water into bulk, are thus explicable on the view that coalescence of the oil globules is possible when the solid protecting film is removed by solution.

(D) The more recent views on emulsification are essentially based on surface tension considerations, due to early investigators such as Plateau [2] and Quincke,[3] later and more fully by Donnan,[4] and then modified so as to admit of general application to both types of emulsion by Bancroft,[5] Clowes,[6] Briggs,[7] and other American chemists.

In 1891 Rachford [8] showed that a purified and neutral oil, when shaken up with dilute sodium carbonate solution, would not form an emulsion. If, however, traces of free fatty acid, *e. g.* oleic acid, be added, emulsification would take place. Later Donnan [9] showed that specially purified and neutral oil gave exactly the same drop number in dilute NaOH as in water,

[1] *Kolloid Zeits.*, **7**, 11 (1910). [2] *Pogg. Ann.*, **14**, 44 (1870).
[3] *Wied. Ann.*, **35**, 589 (1888). [4] *Zeits. Phys. Chem.*, **31**, 42 (1899).
[5] *Journ. Phys. Chem.* (papers in 1912 and 1913).
[6] *Ibid.*, **20**, 407 (1916).
[7] *Ibid.*, **19**, 478 (1915). [8] *Journ. Physiol.* **12**, 72 (1891). [9] *Loc. cit.*

but that when not so purified the oil gave a drop number nearly six times that given in distilled water. Pure hydrocarbons exhibited similar effects, traces of stearic acid at once rapidly increasing the drop number in alkali. Now such an increase in drop number indicates a diminished interfacial tension, due to the presence of soap formed by the union of the soda and fatty acid. Consequently the first principle to be enunciated is, as already remarked, that a decrease in surface tension favours emulsification. Indeed this was considered the *sine quâ non* for emulsification in general, until Pickering obtained concentrated and permanent emulsions using such third agents as basic iron sulphate, which has not any influence at all on surface tension. Also the formation of emulsions of the water-in-oil type was not adequately explained on this theory. Hence the idea was advanced, and is now firmly established, that although surface tension plays a most important rôle in emulsification, the main consideration is that of an adsorption effect, whereby oil particles or water particles, as the case may be, receive a coating or surface film which acts in three principal directions—

(*a*) the interfacial tension is decreased so that the dispersed particles do not coalesce so readily;

(*b*) an electric charge is conferred on the particles, possibly by ionic adsorption, and this charge will cause electric repulsion between the particles;

(*c*) there is a purely mechanical action keeping the particles separated.

According to the adsorption theory, the type of the emulsion obtained is simply dependent on the nature of the adsorbed film, *i. e.* on the nature of the emulsifier employed. Whether this third substance or film is more easily wetted by oil or by water is a most important factor. Consider a film one side exposed to oil, the other to water, the latter wetting it the more easily. The surface tension on the water side is lower than on the oil side, and consequently the film will curve so that the side with the higher surface tension will be concave, and oil drops be formed enclosed in a water medium. This is simply an illustration of the fact that the sphere has the smallest surface for a given volume of oil, and that owing to surface tension there is always a tendency for surface reduction to a minimum. The area of the inner surface of a film is less than that of the outer, consequently the area of the side of the film of higher surface tension is at a minimum compared with that of the lower surface tension.

The adsorbed film may be of various natures. Thus soot wetted more easily by oil than water leads to an emulsion of water in oil. Soaps may lead to both types of emulsion, as shown by Clowes [1]: "Soaps of the monovalent cations Na, K, etc., used as emulsifying agents for oil and water, cause the formation of an emulsion of oil in water, whilst soaps of Ca, Mg, and other di- and tri-valent cations give a reverse type of emulsion."

Another type of stability may be furnished by simple ions alone, the electric charge and the size of the globules then being the main factors. Thus Ellis [2] showed that such an emulsion was comparable to a colloidal suspension, and its stability was not a function of surface tension, but of the interface potential, being a maximum when the contact potential was a maximum.

The present adsorption theory does not recognize any limiting concentrations for oil in water, the dispersion being quite independent of the volumes employed. Rather does it lay emphasis on the fact that emulsification is influenced by—

(i) the mass of the emulsifying agent present;

(ii) the ease with which the emulsifier can be adsorbed at the interfacial separating layer;

(iii) the nature of the ions adsorbed by the resultant film.

Other things being equal, if there is an excess of negative ions adsorbed by a stabilizing membrane formed between the oil and water phases, there will result an oil-in-water type of emulsion; an excess of adsorbed positive ions leads to the reverse type.

Several results, especially those obtained by Clowes, may be quoted to support the views of the adsorption film theory. Thus if an oil is emulsified in a soap solution, it is always found that the ratio between the drop number and the concentration of the soap solution is logarithmic (cf. the log. form of Freundlich's equation). Again, to prove that ions are adsorbed on the interfacial membrane, Clowes [3] took an emulsion containing oil, water, soap, and NaOH. Adding sodium chloride in regular increments up to a concentration of $\frac{N}{10}$ and titrating against hydrochloric acid, he found that "the amount of hydrochloric acid required exhibited an approximately logarithmic relation to the amount of sodium chloride added, and a similar result was obtained by

[1] *Journ. Phys. Chem.*, **20**, 407 (1916).
[2] *Zeits. phys. Chem.*, **80**, 597 (1912).
[3] *Journ. Phys. Chem.*, **20**, 424 (1916).

titrating soap suspensions to which alkali and varying proportions of sodium chloride had been added, but without the addition of any oil, thus showing that the phenomenon in question is attributed to an effect exerted on the soap particles themselves."

It is interesting to note that all emulsifying agents are colloidal in nature. Thus gelatin, casein, albumin, starches, etc., are true colloids. The soaps also furnish colloidal systems, and it is very striking to find that the sodium salts of the lower fatty acids are not emulsifiers, lauric acid being the first in the series to be effective, and that such salts have been found *not* to yield colloidal solutions, whilst the sodium salts of lauric acid and onwards do.[1] Again, in Pickering's work it was soon evident that the finer the state of subdivision of the solids used, the more perfect was the emulsion; but the finer the particles become the more nearly do they approach true colloidal suspensions.

The marked increase in viscosity accompanying emulsification is anticipated by a consideration of the adsorption diaphragm produced. In illustration of this the homogenization of milk or cream may be cited. By forcing milk or cream warmed to 50° or 60° C. through minute orifices under great pressure, often 5000 lbs. per sq. in., the fat globules are so reduced that their diameters are now about $\frac{1}{100}$th of their initial value. When such a cream or milk is allowed to stand, no separation occurs. The surface tension is decreased, a vigorous Brownian movement is exhibited, and a remarkable increase in viscosity takes place. This is due to casein adsorption by the fat globules, so that the milk is much thicker than ordinary milk of the same fat content. Wiegner reduced the average diameter of butter-fat particles in milk from 2·9μ to about 0·27 μ. From viscosity measurements he calculated that in ordinary milk about 2% of the casein is adsorbed, whilst 25% was adsorbed in the homogenized sample. Skim milk cannot be homogenized, since it is nearly fat-free, and the casein present is already in a high degree of dispersion. Homogenized cream cannot be churned into butter; also, it cannot be " whipped," and unless a colloid such as gum tragacanth be added, no permanent foam is produced.

Emulsions in which soap is the emulsifying agent also increase in viscosity, and Bancroft asserts that it is due to the viscosity of the adsorbed surface films of soap. He points out that " if we have an infinite number of very small semi-solid films or

[1] *Comptes rend.*, **146**, 484 (1908).

membranes, we have practically a cellular structure, and consequently the maximum rigidity."

The adsorption film theory of emulsions is readily applicable to the peculiar instances of an emulsion of one type being converted into one of opposite nature, *e. g.* if an emulsion of olive oil in dilute sodium hydroxide be shaken with increasing quantities of calcium chloride, the original oil-in-water emulsion will change over into an emulsion of water in oil, as soon as the concentration of calcium chloride relative to sodium hydroxide exceeds the ratio of 1 : 4. Emulsions of oil in water can be converted into emulsions of water in oil by shaking with salts of Ca, Mg, Fe, and other di- and tri-valent cations, and the change may be prevented, or the reverse change induced, by shaking with a sufficient excess of sodium hydroxide. Clowes emphasizes the fact that " these antagonistic effects are attributable to a balance between cations on the one hand and anions on the other, adsorbed on, or reacting with, soaps or other colloidal constituents of surface films or membranes." Thus with calcium chloride the cation Ca is more readily adsorbed than the Cl anion, whilst in the case of sodium chloride the Cl anion is more readily adsorbed than the Na cation (cf. also Shorter and Ellingworth's results[1]). Clowes illustrates his theory by reference to a honeycomb structure, in which, for example, the water phase is surrounded by the continuous wax walls of the cells. " Corrosion of the walls promotes the continuity or intercommunication of a previously dispersed water phase."

If NaOH is added to an emulsion of water in oil stabilized by a calcium soap, the drop number increases, this increase being in a logarithmic ratio to the amounts of NaOH added. The increase in the number of drops indicates the greater dispersion of the film and its consequent greater permeability to water. Finally, sufficient NaOH will have been added to so increase the number of oil drops that a continuous chain of drops is the result; this indicates that the system is now absolutely permeable to water, the critical point having been passed, and an emulsion of oil in water has been produced.

Clowes followed the conversion of an oil-in-water emulsion to one of opposite type (under the influence of calcium salts) by the microscope, and observed that " the oil globules dispersed in the water are first elongated as the critical point is approached, and Brownian movement is very marked. At the critical point,

[1] *Proc. Roy. Soc.*, **92**, (A), 231–47 (1916).

extremely active movement of large oil-and-water masses is probably due to the existence of two continuous phases. Beyond the critical point the emulsion consists principally of large drops of water surrounded by oil, which are characterized by the fact that they still contain numerous oil globules in rapid Brownian movement. The complete conversion of the system into an emulsion of water in oil at the point at which sodium oleate is entirely converted into calcium oleate, is perhaps more readily recognized by observing the point at which the Brownian movement of these oil globules dispersed in water entirely ceases."

Summarizing the present position, we see that an emulsion of oil in water is produced if the emulsifying agent is a colloid soluble in water, or more easily wetted by water than oil, and an emulsion of water in oil is obtained when the emulsifying agent is an oil-soluble colloid, or is more readily wetted by oil than by water.

It now remains to discuss the physical chemistry of butter and margarine in the light of the modern conception of emulsions.

Cream is usually separated from milk for butter-making by means of a centrifugal separator, taking advantage of the differing densities of the fat and milk serum. Recently quite a new way, introducing adsorption phenomena, has been proposed. Clavel [1] claims that by converting milk into a froth by streaming gases through it, the cream may be obtained by now pouring through a fine sieve. Similarly, separated milk can be made to yield its casein and albumin in the same way. The principle involved, namely, adsorption at a large interface, such as foam or froth presents, is well known, and has been investigated by Ramsden,[2] Zawidski,[3] Benson,[4] Milner [5] and others.

When cream is churned into butter, a reversal of the type of emulsion occurs; cream is an oil-in-water emulsion, whilst butter is a water-in-oil type. Hence churning, which is, of course, excessive agitation, causes *de-emulsification* of the cream. There seems to be a connection here with the observations of many investigators who have found that over-agitation of an emulsion causes it to "break." Cream "breaks" into fat and water, and the subsequent butter only contains water globules by pure mechanical absorption and kneading.

[1] Ger. Pat. 314,090 (1918).
[2] *Proc. Roy. Soc.*, **72**, 156 (1903), and *Zeits. phys. Chem.*, **47**, 336 (1904).
[3] *Zeits. phys. Chem.*, **35**, 77 (1900).
[4] *Journ. Phys. Chem.*, **7**, 532 (1903).
[5] *Phil. Mag.*, **13** (vi.), 96 (1907).

In making a margarine emulsion, it is quite possible that a critical state exists for the production of the most stable emulsion.[1] The writer is investigating this question, and proposes to determine, if possible, the critical speed at which the electric emulsifier (*e. g.* Silkeborg type) must be run to give a really permanent emulsion, all other conditions being fixed.

An emulsion of great stability and permanence is not demanded, as such, in margarine practice. Undoubtedly such an emulsion would yield the best-finished article as regards texture. Since, however, the emulsion is instantaneously cooled in small portions by an ice-water spray or on a chilled drum, no separation or "break" of an emulsion is possible, and hence all that is required is maintenance of agitation or mixing of the oils and milk serum whilst chilling is being conducted.

The preparation of a good margarine emulsion depends on several factors. It is of paramount importance to secure the oil-in-water type, and this is attained by running the oils into the milk, stirring continually. Since milk contains water-soluble colloids (emulsifiers), such a procedure facilitates the emulsification and leads to a stable system. If, now, the temperature be decreased, agitation still being maintained, a very stable system is reached at about 25° C., for here the viscosity effect comes into play, with beneficial results. From such considerations as these the author prefers the bulk churns, for in them the milk can be placed in quantity and the oils run in. No such freedom in the changing of physical factors is given by the newer continuous churns, *e. g.* Silkeborg's electric emulsifier. Blichfeldt's emulsifier allows of more efficiently producing a stable oil-in-milk emulsion.[2]

Several patents have been secured, claiming the use of emulsifying agents for producing fine margarine emulsions.[3] Egg-yolk, and egg-yolk preparations in sesamé oil, have been extensively used. Glycerine, gelatine, starch, albumin, and other colloids have also been recommended. For laboratory work, gelatine is excellent.

The production of a margarine emulsion presents many problems of great interest to the colloid chemist. A very complex physico-chemical system is presented. In the first place, a single oil is not used, but a mixture of perhaps six oils and fats

[1] See Sheppard, *Journ. Phys. Chem.*, **23**, 637 (1919) (influence of agitation on nitro-benzene emulsions).

[2] Eng. Pat. 8227 (1912).

[3] Eng. Pats. 21,626 (1900); 8099 (1903); 9015 (1907); 4412 (1912); 120,836 (1918); 125,172 (1919); 129,165 (1919).

in varying proportions. These constituents do not possess the same ease of emulsification. From drop-number determinations made in the author's laboratory, it is found that the liquid oils (cottonseed, arachis, olive) possess approximately equal emulsifying capacities, differing, however, from those of the melted animal and vegetable fats. It is hoped to publish a full account of this work in due course.

Again, there is the factor due to the presence of solid casein flocks in the sour milk used. On theoretical grounds it may be anticipated that these solid particles help the formation of the margarine emulsion. The lactic acid present is also a notable factor. The author's experiments show this acid to aid the emulsification. Other factors in this complex system are temperature, viscosity, and the colloids in actual solution. Occasionally the emulsion is salted before chilling. From drop-number determinations in the author's laboratory, it has been observed that a concentration of 1% NaCl helps emulsification. This is probably due to the adsorption of chlorine anions at the oil-water interface, whence the caustic soda formed as a result of this adsorption, uniting with the free fatty acids present, *e. g.* oleic, produces a soap which would at once induce emulsification.

Another point of interest in connection with margarine emulsions, is that two types of margarine are possible, viz. : an oil-in-water type, or water-in-oil type. The former may be induced by spraying oil into milk whilst agitating, and the latter by spraying milk into excess of the oil and agitating, in each case effecting instantaneous cooling on a chilling drum. To determine which type one is handling, several methods can be used. One excellent method is based on an extension of Briggs'[1] drop-dilution method. A solid emulsion of water in oil will gradually dissolve if placed in olive oil. A solid margarine of the opposite type would not so dissolve. Sometimes the staining of a margarine with Sudan III and subsequent examination under the microscope will reveal the type of emulsion.

To the physical chemist, margarine technology affords an ample field for research, particularly on emulsion problems. So far, practically no work has been done on *solid* emulsions, of which butter and margarines are interesting, if complex, cases.

[1] *J. Phys. Chem.*, **19**, 478–99 (1915).

CHAPTER IX

BUTTER AND RENOVATED BUTTER

Butter

Butter is a fatty product obtained by separating and subsequently churning the cream from cows' milk, and it contains most of the fat originally present in the milk. The operation of churning causes the cream emulsion to "break," the butter-fat globules coalescing to form granules, which are afterwards kneaded together to produce a mass of apparently homogeneous structure. In addition to fat, butter contains water, casein, and added substances, such as salt and artificial colouring matter.

The composition of butter varies with the conditions of its manufacture. The average of 695 samples of American butter has been officially given as:[1] fat = 82·41%; water = 13·90%; salt = 2·51%; and curd = 1·18%. The following table of mean values is due to Vieth[2]—

Butter.	% Fat.	% Curd.	% Salt.	% Water.
English . . .	86·85	0·59	1·02	11·54
French, fresh . .	84·77	1·38	0·09	13·76
,, salted . .	84·34	1·60	2·01	12·05
Kiel	85·24	1·17	1·35	12·24
Danish . . .	83·41	1·30	1·87	13·42
Swedish . . .	83·89	1·33	2·03	13·75

The fat content of butter usually falls within the limits 83–87%, and that of the curd 0·6–1·5%, whilst the amount of salt can be altered to suit the taste, but usually ranges from 1·5–3·5%.

Normal milk contains about 3·6% of fat, the major portion of which rises to the surface as cream when left to stand. For buttermaking the cream is quickly and efficiently obtained by

[1] U.S. Bur. An. Ind., *Bull.*, **149**. [2] *Analyst*, **15**, 1 (1891).

passing the milk through a centrifugal separating machine. The density of the liquefied fat is about 0·92, whilst that of the milk serum is over unity. The diameters of the fat globules vary between 0·01 mm. and 0·0016 mm. Thick cream contains approximately 56% fat and 39% water, whilst thin cream contains about 29% fat and 64% water. The fat content, however, varies within wide limits, and the percentage of fat is in inverse ratio to the density of the cream. It is generally assumed that the fat globules in milk are surrounded by some kind of protecting envelope, resultant upon adsorption effects. The membrane is probably semi-liquid in nature, and is often referred to as the "slim-membran" of Storch.[1] This "slim-membran" no doubt plays an important part in the process of churning. Elsewhere the author [2] has said: "As to the exact changes occurring when milk-fat is churned into butter, there is still divergence of opinions. The upholders of the "slim-membran" theory argue that the mucoid substance enveloping the fat globules is rubbed off, and the globules thereupon coalesce. Fleischmann inclines to regard the process of churning as being the solidification of superfused fat globules, but this theory is discredited by the fact that the fat globules in milk are rapidly solidified by mere cooling.

It is very likely, however, that there is an adsorption layer of some kind around the fat, and that during churning this layer is continually thinned out by the impacts of the various globules, eventually permitting coalescence to small nuclei, which grow by degrees until that particular moment arrives when the butter particles suddenly become visible. There is still room for considerable physico-chemical research in connection with this long-known, but little-elucidated problem.

The butter nuclei are worked up to a homogeneous mass, which the microscope shows to be a solid emulsion of fat, with fat the continuous medium, an exact reverse of the system occurring in milk.

The physical conditions of churning, especially the temperature, exert a profound influence on the butter, particularly with regard to its moisture content. The main factors to be considered in this connection are the fat content, the acidity, and the viscosity of the cream, and the agitation employed. A cream containing 30–45% of fat, churned at a temperature ranging

[1] *Analyst*, **22**, 197 (1897).
[2] Clayton, *Brit. Assoc. Comm. Colloid Chem., Report II.*, p. 99 (1918).

from 13–18° C., should give good results. As cream ripens lactic acid is formed, the viscosity of the cream diminishes and churning becomes easier. In this connection it is interesting to note that acids tend to make emulsions "break," *i. e.* separate into oil and water, probably by some action on the protective (emulsifying) agent present. Hence the casein is coagulated and precipitated if the acidity is too pronounced, and casein clots may be found in the butter mass. Churning must not be conducted too rapidly and violently, for then the moisture content will be too high, owing to the enclosure of buttermilk within the nuclei. Also churning must be stopped as soon as the butter granules reach the size of small peas, otherwise the granules will coalesce and retain an excess of buttermilk which cannot be washed out again."

Butter may be made from sweet or from sour cream, and the subsequent flavour is intimately connected with the nature of the cream churned. Sweet cream butter, made, that is, from cream which has not been soured, and so contains but a little lactic acid, has a characteristic *low* flavour, often termed the *primary* flavour of butter. Such butter readily becomes rancid, particularly if the cream was not pasteurized previous to churning. Cream which has been soured, either naturally or by inoculation with a culture of lactic acid bacteria, gives a butter of marked flavour, and is the most usual type met with in the trade. The degree of souring determines the intensity and kind of flavour, the butter-fat absorbing certain aromatic compounds which are produced during the souring period.

Chemical Composition of Butter-fat.—The fat obtained by melting butter and separating from the aqueous medium consists of glycerides of several fatty acids. Much discussion has taken place as to the actual composition, and many conflicting analyses reported. Koefed [1] determined the approximate composition of a sample of Danish butter-fat, and his results indicated 91·5% total fatty acids, made up as follows: butyric acid = 1·5%; caproic acid = 2·0%; caprylic acid = 0·5%; capric acid = 2·0%; lauric acid = 8·0%; myristic acid = 22·0%; palmitic acid = 28·0%; stearic acid 2·0%; oleic acids of the composition $C_{15}H_{28}O_4$ and $C_{29}H_{54}O_5$ = 34%.

Browne [2] gave the following as the composition of a sample of butter-fat investigated by him—

[1] *Analyst*, **17**, 130 (1892).
[2] *Journ. Amer. Chem. Soc.*, **21**, 807 (1899).

	% Fatty acids.	% Glycerides.
Insoluble Fatty Acids.		
Dihydroxystearic acid	1·83	1·04
Oleic acid	32·50	33·95
Stearic acid	1·83	1·91
Palmitic acid	38·61	40·51
Myristic acid	9·89	10·44
Lauric acid	2·57	2·73
Soluble Fatty Acids.		
Capric acid	0·32	0·34
Caprylic acid	0·49	0·53
Caproic acid	2·09	2·32
Butyric acid	5·45	6·23

Duclaux [1] found butter-fat to contain from 2–2·26% caproic acid, and 3·38–3·65% butyric acid.

The amount of stearic acid present in the fatty acids of butter-fat has been much disputed. Lewkowitsch [2] examined a butter of which the Reichert-Meissl value was 28·1, and found only 0·49% stearic acid in the total fatty acids present. Hehner and Mitchell [3] also agree that the content of this acid is very low and in many cases *nil*. Siegfeld,[4] as a result of his investigations, concludes that the volatile soluble acids are entirely butyric and caproic, whilst the volatile insoluble acids contain caprylic, capric, myristic, lauric, and perhaps traces of palmitic acid. The solid non-volatile acids were myristic and palmitic, and he points out that he never obtained more than traces of formic, acetic, and stearic acid.

On the other hand, Smedley [5] found from 10–15% of stearic acid in the samples of butter-fat examined, whilst Holland, Reed, and Buckley [6] found from 7–22% of this acid in the total fatty acids of the butter samples investigated. At the same time they observed that the stearic acid content depended on the food of the cow, foods rich in fat, such as beef-tallow or palm oil, leading to an increase in the amount of stearic acid in the insoluble fatty acids of the resulting butter-fat.

Recently Holland and Buckley [7] investigated the fatty acids of butter-fat, employing a direct esterification of the fat and then fractionating the esters obtained. The fat was esterified for

[1] *Comptes rendus*, **102**, 1022 (1886).
[2] *Oils, Fats and Waxes*, 5th ed., Vol. I. 639.
[3] *Analyst*, **21**, 329 (1896).
[4] *Zeit. Nahr. Genussm.*, **24**, 453 (1913).
[5] *Biochem. Journal*, **6**, 451 (1913).
[6] *Analyst*, **21**, 209 (1916).
[7] *Journ. Agric. Research*, **12**, 719 (1918).

twenty-four hours with absolute alcohol charged with dry hydrochloric acid. The esters were purified with ether and anhydrous $MgCl_2$, and distilled from an oil bath. Each fraction then contained two ethyl esters together with some ethyl oleate. Determining the iodine and saponification values for each fraction, they calculated the amount of ethyl oleate algebraically from the saponification value obtained. Thus they were able to determine lauric, myristic, caproic, caprylic, and capric acids. Butyric and palmitic acids were determined by difference, and stearic acid by the Hehner and Mitchell crystallization method.[1] Their final results indicated the following percentage composition of the fatty acids in butter-fat: caproic acid = 1·36; caprylic acid = 0·97; capric acid = 1·83; lauric acid = 6·89; myristic acid = 22·62; butyric acid = 3·15; palmitic acid = 19·23; stearic acid = 11·38; oleic acid = 27·34.

It is believed that the glycerides present in butter-fat are *mixed* glycerides and not simple triglycerides of particular acids. Blyth and Robertson,[2] finding it impossible to separate tributyrin from butter-fat by solution in hot alcohol, argued that a mixed glyceride must be present, since an artificial mixture of triglycerides containing tributyrin could be easily separated. The work of Henriques and Künne [3] on the chlor-iodo compounds of the glycerides of butter-fat also supports this view. Bell [4] claims to have proved the presence of "oleo-palmito-butyrate of glycerol" in butter-fat. Amberger,[5] from experiments on the fractional crystallization of hydrogenated butter-fat from various solvents (alcohol, ether, acetone), showed the presence in butter-fat of such mixed glycerides as butyrodiolein, butyropalmitoolein, and oleodipalmitin. He found that the greater part of the oleic acid in butter-fat is present as a mixed glyceride and not as triolein. Mixed glycerides are also known to occur in other natural fats, for example, lard [6] and mutton-fat,[7] which each contain palmito-distearin, differing, however, in melting-point and crystalline form. Lard also contains about 2% of stearodipalmitin.

The chemical composition of butter-fat is known to vary between fairly wide limits, and is influenced by such factors as the breed of cow, the mean temperature to which it is exposed,

[1] *Analyst*, **21**, 316 (1896).
[2] *Proc. Chem. Soc.* (1889), p. 5.
[3] *Berichte*, **32**, 387 (1899).
[4] *The Chemistry of Foods*, Vol. II. 44.
[5] *Zeit. Nahr. Genussm*, **35**, 313 (1918).
[6] Bömer, *Zeit. Nahr. Genussm.*, **25**, 321 (1913).
[7] Bömer and Limprich, *Ibid.*, **25**, 354 (1913).

the kind and quantity of the food, and the period of lactation. Such influences are reflected in the usual analytical "constants," of which the following are recognized for butter-fat—

Analytical constant.	Mean value.	Limits.
Specific gravity at 15·5° C.	0·938	0·936–0·942
Melting-point	31° C.	28–34° C.
Solidifying-point	21° C.	19–24° C.
Solidifying-point of fatty acids	35° C.	33–37° C.
Specific heat	0·507	0·485 at 30° C. 0·53 at 60° C.
Refractive index 40° C. (Z.B.)	40°	38–42°
Iodine value	33	25–50
Saponification value	227	221–233
Valenta number	45	40–50
Reichert-Meissl value	28	25–30
Polenské value	2·3	1·7–2·9
Kirschner value	—	20–26
Unsaponifiable matter	0·35	0·3–0·45

The analysis of butter includes determinations of water, fat, casein and salt, and further, an examination of the fat for indications of adulteration. A full account of the methods of analysis is given in Chapter X, where the analysis of butter and margarines is considered together in detail.

Renovated Butter

"Renovated" butter, also known as "process" butter or "milk-blended" butter, is a product obtained by the clarification and subsequent re-working of butters of poor quality, or which have deteriorated during transit or storage. Even rancid butter can be made saleable after such treatment. By the processes included in renovation the obnoxious odours and flavours practically disappear, and the finished article is quite uniform and wholesome when put on the market.

"Renovated" butter has been made in large quantities in the United States since 1883, where regular Government supervision and inspection of the renovated butter factories are maintained. An Act of Congress of May 9, 1902, sets out the legal definition of "renovated butter" in the clauses—

"This grade or kind of butter may be made from one or more lots or parcels of butter which has been or have been 'subjected to any process by which it is melted, clarified, or refined and made to resemble genuine butter, always excepting 'adulterated butter' as defined by this Act.

"The butter, to be subject to this definition, must have been

melted—that is, so affected by heat as to become of sufficient fluidity to move in a continuous stream of even consistency from one vessel to another by pouring or pumping, because butter cannot be "clarified or refined" unless it be melted to that degree.

"The butter must, besides melting, have been subjected to some process by which it is "clarified or refined." Butter, or melted butter, may be clarified or refined by skimming, aerating. washing, and other processes, through the action of heat, cold, agitation or motion, or rest.

"Butter thus melted and clarified or refined becomes an oil or fat almost free from taste and odour. To be again 'made to resemble genuine butter,' it must have restored to it the butter characteristics or similitude of texture, granulation, and flavour. For this purpose the processed or renovated butter is usually mixed with milk, or skim milk, or buttermilk, or cream, sweet or sour, and granulated by cooling. It may or may not have common salt or artificial colouring added. To 'resemble genuine butter' the article must have passed through these or other processes subsequent to melting, so that it looks, smells, and tastes like 'butter,' having a similar appearance, consistency, texture, and flavour."

Renovated butter containing any substances not found in ordinary butter, or whose water content exceeds 16%, is classed as an "adulterated butter." Cleanliness is most essential at every stage during renovation, and even then the keeping qualities of the product are much inferior to those of ordinary butter.

Renovation is usually conducted as follows: the butter to be treated is melted and the oil and water allowed to separate into layers. The water containing curd and salt is run off, or in some factories separated in centrifugal separators. The butter, as oil, is washed with water and again separated. Then follows the operation of "blowing," *i. e.* aerating by blowing air through. Finally, suitably soured milk is added, an emulsion made, and this rapidly chilled with a spray of ice-cold water, thus simulating the usual butter granules. By rolling, kneading or working, the mass is made to assume a butter-like texture, and has its moisture-content brought within the legal limit.

Chemically, renovated and genuine butters are practically identical, and physical tests must be employed to differentiate between them. The only stage in renovation where the butter-fat might conceivably undergo chemical change is "blowing," where possible oxidation changes might occur. However, such

action does not take place, probably due to the low temperature employed. The following table, due to Crampton,[1] summarises the results of seventy-five analyses of renovated butters, and shows the impossibility of recognizing process butter by purely chemical analyses—

	Maximum.	Minimum.	Mean value.
	Per cent.	Per cent.	Per cent.
Fat	88·88	86·80	82·05
Curd	2·65	0·77	1·47
Ash	7·49	0·97	2·85
Water	23·17	8·01	14·44
Specific gravity $\frac{40°}{40°}$ C.	0·9124	0·9093	0·9106
Refractive index at 25° C.	1·4619	1·4600	1·4608
Reichert-Meissl value	31·82	25·42	29·15
Valenta value	50·0° C.	33·5° C.	41·6° C.
Crismer value	54·80° C.	43·20° C.	49·05° C.
Acidity	13·40	1·72	6·57
Iodine number	41·15	34·20	36·78

The specific gravity was determined on fifty-eight samples, the Crismer, Valenta, and acidity values on fifty-four samples, the iodine number on twenty-five samples, and the other values on seventy-five samples.

To test the possible changes due to "blowing," Crampton[2] determined the usual constants of a fresh butter prior to allowing it to become rancid, and then when rancidity and mouldiness were evident, he conducted renovation of the sample on a laboratory scale. His analytical results are tabulated thus—

FAT VALUES

	Same butter.		
	Fresh.	Rancid.	Renovated.
Specific gravity $\frac{40°}{40°}$ C.	0·9106	0·9117	0·9117
Refractive index at 25° C.	1·4612	1·4613	1·4614
Reichert-Meissl value	27·97	27·27	27·46
Acidity (c.c. $\frac{N}{1}$ alkali per 100 gr. fat)	—	4·28	4·28
Hehner value	88·94	85·43	85·23
Valenta value	44·75°	49·0°	48·0°
Crismer value	58·5°	49·7°	49·7°
Saponification value	217·3	217·3	214·8
Per cent. soluble fatty acids	4·63	3·77	3·89

[1] *Journ. Amer. Chem. Soc.*, **25**, 358–66 (1903). [2] *Loc. cit.*

Crampton observes[1]: "It will be seen that the fat values of the butter after renovation differ less from those of the butter before renovation than the latter differ from the values of the fresh butter." Any changes that may have occurred in the composition of butter-fat during renovation are so slight as to pass recognition.

Detection of Renovated Butter.—Of the various tests proposed, none is infallible, and all fail to respond at times. Most reliance can be placed on the Brown-Taylor-Richards test,[2] which is very definite in its indications. Another test which is also quite good is the Waterhouse test.[3]

The Brown-Taylor-Richards test depends on the appearance of the butter when viewed under a polarization microscope. When butter is renovated, the fat assumes a semi-crystalline structure, induced by the melting and rapid cooling, whilst ordinary butter contains its fat in an isotropic or non-crystalline form. Consequently under the polarization microscope fresh butter shows a dark field with few or no polarizing crystals, whilst renovated butter exhibits many such crystals. Margarines, owing to the method of manufacture, behave similarly to renovated butter. Sometimes the usual dark field given by fresh butter shows a few bright specks due to crystals of common salt and preservatives, but an examination with the ordinary microscope will then reveal them; experience in this test soon teaches the analyst to avoid mistaking such bright spots for crystalline particles of fat.

To conduct the test, a small fragment of butter is placed on a glass slide, covered with a micro-circle, and examined under a polarizing microscope at 120–150 magnifications. If a selenite plate be placed between the slide and lower nicol, pure butter will show a field of uniform colour (*e. g.* blue), whereas renovated butter will show a coloured field (*e. g.* blue) mottled throughout with colours (*e. g.* yellow). Care must again be taken to avoid errors due to particles of salt or preservatives.

Amongst other tests proposed for the detection of renovated butter are: (1) the "spoon test," which consists in heating about 1 gram of the sample over a free flame; pure butter froths and gently browns, whilst renovated butters and margarines

[1] *Loc. cit.*, p. 364.

[2] Crampton, *Journ. Amer. Chem. Soc.*, **22**, 703–5 (1900); Hummel, *Ibid.*, **22**, 327–9 (1900); Hess and Doolittle, *Ibid.*, **22**, 150–2 (1900).

[3] Patrick, *Proc. Assoc. Official Agric. Chemists* (1901), p. 126; Deguide, *Journ. Pharm. Chim.*, **16**, 372 (1902).

spurt and hiss. (2) The Hess and Doolittle test,[1] which depends on the appearance of the curd. Whilst fresh butter gives a curd of a cohesive, gelatinous nature, renovated butter gives a flaky, or granular, non-cohesive curd (coagulated casein). Hess and Doolittle recommend melting the sample in a beaker, decanting the fat, and washing the curd free from fat with ether. The curd is then spread on a glass slide, and, when dry, examined by a lens magnifying three to six diameters. They claim that "the curd from true butter will have an amorphous, non-granular appearance, while the curd from "process" butter has a very coarse, curdy appearance." The present writer's experience is that this claim is not rigidly correct, and is by no means so consistent as the polarization-microscope test.

[1] *Journ. Amer. Chem. Soc.*, **22**, 150–2 (1900).

CHAPTER X

ANALYSIS OF BUTTER AND MARGARINE

THE analysis of butter and margarines includes determinations of moisture, salt, preservative, casein and fat. Occasionally the nature of the added colouring matter (if any) is also required. To obtain these results, the methods of analysis of butter and margarine are practically the same, and will be considered together. In the case of margarines an analysis of the composition of the oils and fats employed is required, and in the case of an adulterated butter, the nature and extent of adulteration. These two cases will be discussed separately.

Water.—A fair sample of the butter or margarine is taken by dividing the bulk sample, and taking three or four pieces from different parts, *e. g.* the middle and opposite corners. These pieces are put into a wide-mouthed stoppered bottle (6 ozs. is a convenient size), melted at about 40° C. (preferably in a thermostat), and then shaken until practically solid. The moisture content may then be determined in several ways—

1. Five grams are weighed into a weighing bottle, about 2 inches deep and 1½ inches wide. This is placed in a steam or electric oven at 100° C., shaken at intervals, and weighed to constant weight. Two to three hours usually suffices for this estimation.

2. For rapid routine or control work, 10 grams are weighed into a nickel or aluminium crucible, together with about 0·2 gram of fine pumice powder. The crucible is heated on a sand-bath until frothing ceases, and the curd becomes just slightly browned. The crucible is then cooled in a water-tray and reweighed. This method cannot be used if the solids not fat and salt have also to be determined on the same sample.

Fat.—The residue in either of the above methods is extracted with ether or chloroform, at least four or five times, decanting the solvent very carefully after each extraction. The residue is then dried to constant weight in the water-oven, and the loss

in weight represents the fat content. If desired as a check, the solvent may be evaporated and the fat estimated directly.

Solids not Fat.—The residue contains solids not fat, together with salt and preservative. Estimation of the salt and preservative gives the solids not fat by difference.

Curd.—The residue after fat extraction is treated with warm water and filtered, the filter and residue being well washed. The curd remains undissolved and is conveniently estimated by simple drying and weighing. If a more accurate determination is required, the nitrogen in this residue is estimated by Kjeldahl's process, employing the factor 6·38.

Salt.—The aqueous filtrate consequent upon the extraction with water in determining solids not fat is titrated with standard silver nitrate solution, using K_2CrO_4 as indicator. It is usual to employ silver nitrate solution so standardized that 1 c.c. = 0·005 gram of sodium chloride.

Lactose.—This is only occasionally required to be determined, and serves to indicate the addition of "milk-powder" or "dried-milk" to butter and margarine. For its estimation an aliquot portion of the aqueous filtrate obtained for the chloride estimation is titrated against Fehling's solution, accurately standardized against a solution of pure lactose containing 0·25 gram per 100 c.cs. The maximum content of lactose monohydrate in a butter or margarine should not exceed 0·5%, unless dried milk has been added.

Preservatives.—The usual preservative in butter and margarine is boric acid, or a mixture of boric acid with borax. Other reagents employed are salicylic acid, benzoic acid, fluorides, β-naphthol, sulphites, nitrates, and formalin—

Boron Compounds.—Boron preservatives may be qualitatively detected by taking a small, pea-sized piece of butter or margarine and melting in a white flat dish with a drop of concentrated hydrochloric acid. The melted fat is stirred, and five or six drops of an alcoholic solution of turmeric stirred in. As the edges dry, a characteristic salmon-red colour is formed, which, on addition of a little ammonia, passes to purple green.

Boron compounds are quantitatively estimated by a convenient and rapid method due to Richmond and Harrison.[1] The results are expressed in terms of boric acid, H_3BO_3, the legal limit of which is 0·5%. The method is conducted as follows—

Twenty-five grams of the sample are weighed into a beaker

[1] *Analyst*, **30**, 250 (1905).

and gently warmed to melting, when 25 c.cs. of warm water are added and the contents well mixed. The mixture is then allowed to settle, and from the lower aqueous layer 20 c.cs. are withdrawn by a pipette. To this 5 c.cs. of a 1% solution of phenolphthalein in strong alcohol are added, then an excess of $\frac{N}{10}$ NaOH (about 2 c.c.), and the mixture raised to boiling. Whilst still boiling, $\frac{N}{10}$ H_2SO_4 is added until the liquid is only very faintly alkaline. Now add 2 grams of mannitol or 30 c.cs. of glycerol, cool the mixture, and titrate with $\frac{N}{10}$ NaOH until faintly pink. The percentage of boric acid is given by substituting in the formula—

$$(x-y) \times 0{\cdot}0062 \times 5$$

where x = c.c. of $\frac{N}{10}$ NaOH required in the final titration, and y = blank for mannitol or glycerol employed.

1 c.c. of $\frac{N}{10}$ NaOH = 0·0062 gram boric acid.

Salicylic Acid.—This substance may be detected by adding aqueous ferric chloride to melted butter or margarine; on shaking a violet coloration is produced. For its estimation, the most convenient method is that of Bolton and Revis [1] (modification of the methods of Fellenberg, Harry, Mummery, Revis and Payne): 10 grams of the sample are extracted with boiling alcohol (20 c.cs.) three times, pouring each extract through a small filter. To the combined extracts add 150 c.cs. water, neutralize with $\frac{N}{10}$ NaOH, and distil off 60 c.cs. The residue is treated with 10 c.c. of normal sodium citrate (70 gram citric acid made neutral with NaOH and diluted to 1 litre), and then 10 c.cs. basic lead acetate solution (made by boiling 430 grams normal lead acetate and 130 grams of recently-ignited litharge with 1 litre of water; cool, allow to settle, syphon off the clear liquor and dilute to give a density of 1·25). Now add 10 c.cs. of $\frac{N}{1}$ NaOH, 5 c.cs. of $\frac{N}{1}$ HCl, and 40 c.cs. of saturated NaCl solution, dilute to 250 c.cs. and filter. Of the filtrate 200 c.cs. are extracted with ether after making acid with sulphuric acid; this extracts the salicylic acid, which

[1] *Fatty Foods* (Churchill, London), p. 111.

is brought into aqueous solution by washing the ether in a separating funnel with 20 c.cs. water, adding $\frac{N}{10}$ NaOH until the aqueous layer is just alkaline to phenolphthalein. This is run off, and the ether twice washed with 20 c.cs. lots of water; the total aqueous extracts are made up to 100 c.cs. The salicylic acid is estimated colorimetrically in Nessler glasses, against a standard solution of salicylic acid (0·01 gram per litre), employing iron alum indicator (1 gram in 500 c.cs. water, boiled down to 100 c.cs. and filtered. Two c.cs. are added to 50 c.cs. of the salicylic acid solutions in a Nessler glass).

Benzoic Acid.—About 10 grams of butter or margarine are digested with 10 c.cs. alcohol for half an hour. Add two drops dilute sulphuric acid and cool. Decant the alcohol into a separating funnel, dilute with water, add 0·5 c.cs. of dilute sulphuric acid, and extract with 20 c.cs. ether. Allow to separate, wash the ether several times with 5 c.cs. water, and after adding 10 c.cs. of water and 1 drop of phenolphthalein solution, titrate to neutrality with barium hydroxide (saturated solution); the mixture is well shaken, and barium hydroxide added carefully, so that the pink tinge is just permanent. The pink aqueous layer is filtered into a small porcelain basin, evaporated to 1 or 2 c.cs., filtered into a test-tube, and very dilute acetic acid (1 in 100) added by drops so as to just discharge the pink tinge. If now freshly-prepared and neutral ferric chloride be added (1 or 2 drops), a *flesh-coloured* turbidity or precipitate will indicate *benzoic acid. Salicylic acid,* following the same treatment, yields a *violet* coloration.

Fluorides.—Melt 100 grams of the sample in a beaker, pour into a separating funnel, and wash out the beaker twice with light petroleum (25 c.cs. each) and twice with water (5 c.cs. each). After settling, run off the aqueous layer into a platinum basin, make alkaline with sodium carbonate, evaporate to dryness, and ignite until nearly white. (A blank test on a pure butter is simultaneously conducted.) Stir up the residue with distilled water, and add an excess of 20% aqueous calcium chloride solution (about 20 c.cs.), raise to boiling, and add an excess (about 20 c.cs.) of 20% aqueous sodium carbonate solution. Filter; dry the filter and contents in the platinum basin, and ignite. To the resultant ash add 30 c.cs. of 20% acetic acid, cover with an inverted funnel, and digest for fifteen minutes on a sand-bath. Wash the contents of the basin on to a small filter with hot acetic acid solution, dry the filter, and ignite in a tared platinum

crucible; weigh the ash. Fluorides may now be *qualitatively* looked for, by adding a few drops of concentrated sulphuric acid, covering the crucible with a watch-glass whose surface has been waxed over, and scratches made in the wax surface. The watch-glass is kept cooled with water, and the crucible is gently heated for at least thirty minutes. The watch-glass is then removed, the wax cleaned off, and etched-in scratches looked for. Fluorides being thus detected, their *quantitative* determination is carried out as follows: the fluorides are converted into sulphates by very carefully igniting the platinum crucible in an inclined position; by re-weighing, the amount of sulphates is obtained, and, assuming the residue consists entirely of $CaSO_4$ derived from CaF_2, the amount of fluorides is given by multiplying the weight of calcium sulphate by 0·5735. The residue, obtained by running a blank test, is usually very small, and is subtracted from the final weight of $CaSO_4$ before converting into terms of CaF_2.

This method is due to Hehner, who has pointed out that much boric acid interferes with the fluoride test as applied directly to the ash of a butter or margarine, owing to the volatility of borofluorides; hence the need for the treatment with $CaCl_2$ and Na_2CO_3, which can be omitted if boron compounds are proved absent.

β-Naphthol.—The aqueous layer from melted butter or margarine is mixed with a suspension of diazotized naphthionic acid, when, in the presence of β-naphthol, an *immediate crimson coloration* is produced. (Pure butter also responds to this test, but only gradually.) Less than 0·02% of β-naphthol can thus be detected. Salts of the β-naphthol-sulphonic acids (*e. g.* abrastol), which are also preserving agents, are likewise detected by this test.

The reagent is conveniently prepared by digesting 0·2 gram of 1-4-naphthionic acid with 10 c.cs. of 50% alcohol. The mixture is chilled in ice-water, 1 c.c. of sulphuric acid (1 : 3) added, and then very gradually 10 c.cs. of 10% aqueous KNO_2. Allow to stand for about five minutes, and then filter the yellow mixture, washing the precipitate with a little water, and then taking up in 5 c.cs. of water.

Sulphites.—Fifty grams of the sample are melted in a beaker and poured into a separating funnel; the beaker is washed out with 50 c.cs. of chloroform, which is also run into the separator. Allowing for the moisture-content of the sample, water is added, in sufficient quantity to amount to 50 grams of water altogether,

Shake and allow to settle, when 25 c.cs. of the aqueous layer are run off (corresponding to 25 grams of the initial sample). Acidify with dilute HCl, raise to boiling, and pass the SO_2 which is evolved into $\frac{N}{10}$ iodine solution. By titration with standard sodium thiosulphate the amount of iodine used up is estimated, from which the amount of SO_2 is calculated, since 254 parts of iodine convert 64 parts of SO_2 into sulphuric acid.

Nitrates.—Nitrates may be detected in butter or in margarines by the diphenylamine reaction. About 0·01 gram of diphenylamine is placed in a porcelain basin together with 1 c.c. of pure sulphuric acid, and well stirred. A few drops of the aqueous extract of the butter or margarine are now poured down the sides of the basin, whence they flow over the surface of the acid. Nitrates develop an immediate blue colour, whose depth of tint may be utilized for a colorimetric estimation against a standard potassium nitrate solution (1·85 grams in 1 litre water; dilute 30 c.cs. to 1 litre for immediate use; 1 c.c. = 0·00003 gram N_2O_5).

Formalin.—An exact determination is impossible, since formalin combines with protein matter. It may be detected in the aqueous layer of melted butter or margarine by applying Hehner's test, viz. to 5 c.cs. in a test-tube add 5 c.cs. water; add 1 c.c. of 91% sulphuric acid so as to form a layer at the bottom. If formaldehyde is present, a very characteristic violet-blue ring will form at the junction of the two liquid layers. This method readily detects 1 part formaldehyde in 200,000 parts of the sample. Formaldehyde is very seldom used in the preservation of butter or margarines.

Colouring Matters.—Both natural and artificial dyes are used for colouring margarines and, to some extent, butter. Thus annatto, carotin, turmeric, saffron, and coal-tar dyes are all more or less in use, though the coal-tar dyes are rapidly becoming the most widely employed, whilst annatto is open to objection on the grounds of its method of preparation. Doolittle's [1] test for easily distinguishing between annatto and coal-tar colours is now [2] carried out as follows: the clear, melted fat (about 2 c.cs.) is dissolved in a little ether in two test-tubes. To one is added an equal volume of concentrated HCl (the original test was dilute, 1 in 3, HCl), and to the other, an equal volume of dilute (1 in 10) KOH. After shaking, and allowing to stand, the alkaline aqueous

[1] U.S. Dept. Agric. Bur. of Chem., *Bull.*, **65**, 152.
[2] Lubs, *Journ. Ind. Eng. Chem.*, **10**, 436 (1918).

layer will be found to have become yellow if annatto is present, whilst a reddish acid aqueous layer ensues from the presence of azo-dyes.

In the United States artificial colouring matters are prohibited, and such oils or fats as mustard oil and palm oil are employed to colour the margarine.

Various schemes, based on the colour reactions with mineral acids, have been proposed to systematically detect added colouring matters. Thus Leeds [1] describes the following tests—

Colour.	Sulphuric acid.	Nitric acid.	Nitric and sulphuric acids.	Hydrochloric acids.
Annatto	Indigo blue to violet	Blue, becoming colourless	Same	No change or brownish.
Turmeric	Pure violet	Violet	Violet	Violet to original colour on evaporation.
Saffron	Violet to cobalt blue, changing to red-brown	Light blue to light red-brown	Same	Yellow to dirty yellow.
Carrot	Amber-brown	Decolorized	Same, with NO_2 fumes and odour of burnt sugar	No change.
Marigold	Dark olive-green	Blue, changing at once to dirty yellow-green	Green	Green to yellowish green.
Safflower	Light brown	Partly decolorized	Decolorized	No change.
Aniline yellow	Yellow	Yellow	Yellow	Yellow.
Martius yellow	Pale yellow	Yellow, reddish ppt. magenta at margin	Yellow	Yellow ppt. treated with NH_3 and ignited deflagrates.
Victoria yellow	Partly decolorized	Same	Same	Same. Colour returns on neutralization with ammonia.

Lubs [2] has published a comprehensive scheme for the "Detection of Colour added to Butter or Margarine." In an improved form of the Low test,[3] 20 grams of fat are diluted with 20 c.cs. of petrol ether, which is then shaken in a separating funnel with 10 c.cs. of acid mixture (1 vol. conc. sulphuric acid and 10 vols. of

[1] *Analyst*, **22**, 150 (1887). [2] *Journ. Ind. Eng. Chem.*, **10**, 436 (1918).
[3] *Journ. Amer. Chem. Soc.*, **20**, 889 (1898).

glacial acetic acid, 99·5% strength). Pure butter does not colour the acid layer, but azo-dyes cause immediate coloration. Thus—

Red indicates .	Yellow OB, yellow AB, Sudan I, Sudan II, aniline yellow, and butter yellow.
Blue. .	Sudan III.
Yellowish-brown	Sudan G, and aniline-azo-phenol.

The following are the principal oil-soluble colours, and their chemical components—

Yellow AB. . .	aniline and β-naphthylamine.
Yellow OB. . .	*o*-toluidine and β-naphthylamine.
Aniline yellow . .	aniline and aniline.
Butter yellow . .	aniline and dimethylaniline.
Spirit yellow R. . .	*o*-toluidine and *o*-toluidine.
Benzene-azo-phenol .	aniline and phenol.
Sudan I. . . .	aniline and β-naphthol.
Sudan II. . . .	xylidene and β-naphthol.
Sudan III. . . .	amido-azo-benzene and β-naphthol.
Sudan G. . . .	aniline and resorcin.
Sudan brown . .	α-naphthylamine and α-naphthol.
Carminaph Garnet .	α-naphthylamine and β-naphthol.

Lubs has devised a table for identification tests, depending on the use of acid and alkaline reagents, which the writer can recommend as giving satisfactory results. Before extraction with alkali or hydrochloric acid, these reagents are diluted with an equal volume of ethyl ether; before using the sulphuric-acetic acid reagent, dilute it with an equal volume of petrol ether.

LUBS' SCHEME

Dye.	1 conc. HCl 4 water.	Conc. HCl.	1 % NaOH.	1 H_2SO_4 (conc.) 10 glacial acetic acid.
Aniline-azo-phenol	*Nil*	Yellow	Yellow	Yellow-brown.
Sudan G . .	*Nil*	Brown	Brown	Yellow-brown.
Sudan I . .	*Nil*	Very faint red	—	Cherry-red.
Sudan II . .	*Nil*	Very faint red	—	Violet-red.
Sudan III . .	*Nil*	*Nil*	*Nil*	Blue.
Butter yellow .	Red	Red	—	Red.
Aniline yellow .	Red	Red	—	Brown-red.
Yellow OB . .	*Nil*	Red	—	Red.
Yellow AB . .	*Nil*	Red	—	Red.
Amino-azo-α-naphthalene	*Nil*	Violet	—	Violet.

For the systematic separation and identification of these oil-soluble colours, see Mathewson, Bureau of Chemistry, *Bull.*, **137**, 54 (1910), and *Bull.*, **448**, 45 (1917).

As a special test for annatto, Lubs recommends the following: 30 grams of fat are warmed with 60 c.cs. of 2% NaOH, and filtered through a warmed filter. The filtrate is returned and repeatedly filtered for three or four hours. The fat and alkali are then washed from the filter-paper, and a solution of stannous chloride containing a little hydrochloric acid run through, when a red coloration will indicate annatto.

Geissler [1] proposed fuller's earth as an absorbent of azodyes from a fat, as a means of detecting such colours, but many azodyes fail to respond to his test. A trustworthy and comprehensive scheme for detecting artificial colours in butter and margarine is that of Cornelison,[2] which consists in shaking 10 grams of the dry melted fat with 10–20 grams of glacial acetic acid (99·5%), when the acid layer is drawn off, and the colour noted. Then to portions of this acid extract (5 c.cs. each) are added a few drops of strong nitric acid, sulphuric acid, and sulphuric acid together with ether. The scheme is as follows—

CORNELISON'S SCHEME

Colouring matter.	Colour of acetic extract.	Conc. HNO_3.	Conc. H_2SO_4.	Conc. H_2SO_4 + ether to clear.
Pure natural butter.	Water white	Water white	Faint pink after time	Water white.
Sudan I .	Decided pink	Strong pink	Strong clear pink	Pink.
Butter yellow	Very faint pink	Faint pink	Faint pink	Faint colour.
Ceresin orange G (Casella).	Greenish-yellow (strong)	Acid yellow, oil globule salmon-pink	Acid yellow, oil globule salmon-pink	Brownish-yellow.
Yellow OB .	Faint light yellow	Acid faint pink, oil globule salmon-pink	Acid faint pink, oil globule salmon-pink	Pink.
Yellow AB .	Warm ochre-yellow (weak)	Pink, fat colourless	Brown-pink, oil faint pink	Pink..
Annatto .	Dull yellow	Little change	Faint pink after time	Very faint yellow.
Curcumin .	Intense green-yellow	Dull ochre-yellow	Strong pink	Yellow.
Carrot . .	Very faint green-yellow	Faint yellow	Faint pink after while	Very faint yellow.

[1] *Journ. Amer. Chem. Soc.*, **20**, 110 (1898). [2] *Ibid.*, **30**, 478 (1908)

Occasionally it becomes necessary to examine a margarine for such oils as palm oil and mustard oil, which may be present as colouring materials, in place of artificial colours. Palm oil may be detected by the Liebermann-Storch method, in which the filtered fat, at a temperature below 70° C., is mixed with an equal volume of acetic anhydride, and then shaken after the addition of one drop of sulphuric acid (S. G. 1·53). On standing, the lower layer which separates out, will assume a greenish-blue colour, in the presence of palm oil. This colour in transient, and any changes occurring later are to be disregarded.

A method for detecting small quantities of palm oil in margarine, based on Halphen's reagent for detecting rosin oil in mineral oils, has been given by Crampton and Simons:[1] 100 c.cs. of the fat are dissolved in 300 c.cs. of petrol ether and shaken with 50 c.cs. of 0·5% KOH. The aqueous layer is drawn off, made acid with hydrochloric acid, and shaken with 10 c.cs. of carbon tetrachloride. The carbon tetrachloride solution is separated, and part of it tested with the following reagent: 2 c.cs. of a mixture of 1 part crystallized phenol in 2 parts CCl_4 added to it in a porcelain crucible, then 5 drops of hydrobromic acid (sp. gr. 1·19) and the contents mixed by gentle agitation. The almost immediate development of a *bluish-green* colour indicates palm oil.

According to Gill[2] this reaction is essentially a test for " carotin rather than for palm oil, and may be given by butter, oleo oil, or sesamé oil—ingredients ordinarily found in oleomargarine." Consequently this test for palm oil in connection with margarines must be regarded with suspicion until further work is published clearing up the question.

Mustard oil is detected by saponifying the margarine fat with alcoholic potash in the presence of a bright piece of silver. The presence of mustard oil will be indicated if the metal becomes tarnished.

II. Fatty Portion

The analysis of the fatty portion of butter and margarine, in the former case to detect adulterations, in the latter to ascertain as far as practicable the nature and amount of the constituent oils and fats, includes a determination of the refractive index, melting-point, iodine value, saponification value, Reichert-

[1] *Journ. Amer. Chem. Soc.*, **27**, 270 (1905).
[2] *Journ. Ind. Eng. Chem.*, **9**, 136 (1917).

Polenské-Kirschner values, and Valenta value. Other determinations sometimes made in margarine analyses include the Blichfeldt estimation, titer test, and Avé-Lallement numbers.

Refractive Index.—The refractive index of a pure substance under given conditions is a physical constant depending on the alteration of the velocity of light in air when the light is passed into the medium under examination.[1] Various glycerides possess different values of refractive index, and consequently oils and

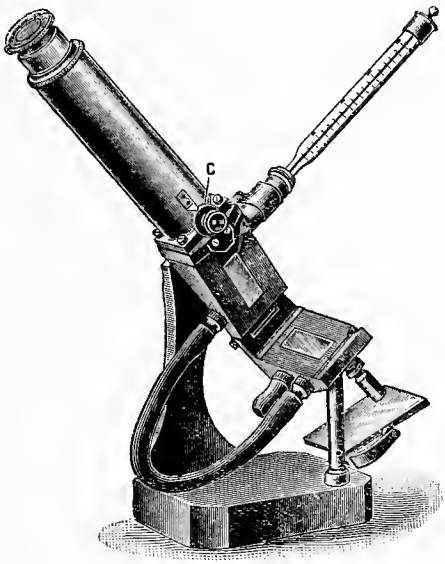

FIG. 16.

From Thorpe's *Dictionary of Applied Chemistry* (Longmans, Green & Co.)

fats also have values, constant over a small range, which admit of rapid and accurate determination by means of a refractometer.

Several types of refractometer are in use, but for the examination of edible oils and fats, the butyro-refractometer (see Fig. 16) is commonly employed, and determinations are made at 40° C. When necessary a higher temperature can be used, and the value calculated to 40° C. by adding 0·55° for each degree (Centigrade) above 40° C. The butyro-refractometer is essentially a double prism maintained at constant temperature when in use. Between the prisms is placed a thin film of the oil under examination, and light

[1] Cf. Philip, *Journ. Soc. Chem. Ind.*, **38**, 139, T (1919).

is projected into the prisms, thus penetrating the oil film. By means of a fixed telescope, the coloured border-line of the total reflection is examined. The objective of the telescope may be adjusted by the micrometer screw, C. In the focal plane of the objective is placed an engraved scale, which is intercepted vertically by the border-line of total reflection, and the field of view is sharply divided into a light and a dark section respectively. The prism is specially constructed so that when butter-fat is examined in daylight, owing to achromatization, a *colourless* border-line is seen. With other oils and fats the border-line has a coloured fringe. The sodium yellow flame is specially recommended as the source of light in the refractometry of oils and fats, the border-line being then much more distinct and intense. The scale is an arbitrary one, but readings thereon may be readily converted into true refractive indices $[n]_D$.[1]

Thorpe [2] examined 371 samples of English butter, and found the refractive index to range from 37·3–43·0 at 45° C. Dutch butters have shown values ranging from 42·0–47·6. In the case of margarine-fat, values over wide ranges may be obtained, since the refractive index is directly calculable from the percentages of the individual oils and fats present, and their particular constants. Readings from 37·0–58·0 at 40° C. are usually obtained for margarines, typically vegetable margarines giving the lowest readings.

Melting-point.—The melting-point of butter-fat ranges from 28–34° C. Margarines and other substitutes for butter are constituted so as to yield a product melting at 22–30° C. The melting-point is purposely advanced during the summer months. In margarine analysis the melting-point of the fat is helpful, since it is practically true to values calculated from percentage composition. For routine work an accurate and simple method of determining the melting-point is to introduce the fat into a capillary tube, which is then attached to a thermometer bulb. This is suspended in a beaker of water, the temperature of which is gradually raised; the temperature at which the fat melts to a clear liquid is then noted.

Iodine Value.—The iodine value of an oil or fat is the percentage of iodine with which it will combine, and is a measure of the *unsaturated* carbon linkages in the constituent glycerides. Pure saturated fatty acids and glycerides have no iodine value.

[1] Roberts, *Analyst*, **41**, 376 (1916).
[2] *Journ. Chem. Soc.*, **85**, 254 (1904).

Of the several methods proposed for determining this constant, that of Wij [1] is rapid and accurate. The author has found this method, using the quantities recommended by Dubovitz,[2] to yield consistently concordant results. In a litre of glacial acetic acid 8·5 grams of iodine are dissolved, together with 7·8 grams of iodine trichloride. An accurately standardized $\frac{N}{10}$ solution of sodium thiosulphate is also required.

From 0·1–0·5 gram of the oil or fat (the quantity depending on the degree of unsaturation of the sample under examination) is accurately weighed into a perfectly clean, dry flask of about 200 c.cs. capacity. Solution is effected by adding 5 c.cs. of carbon tetrachloride, dried over calcium chloride; 10 c.cs. of the Wij solution are added from a dry pipette, the flask is closed with a stopper and allowed to stand thirty minutes. To the contents are then added 5 c.cs. of 10% KI solution and 50 c.cs. of water. Titration is conducted with $\frac{N}{10}$ sodium thiosulphate solution, using 10 drops of 1% starch solution as indicator. A blank test, omitting the fat, is also made simultaneously. The iodine value is calculated by the following formula—

$$\text{iodine value} = \frac{(x - y) \times 0{\cdot}012692 \times 100}{w}$$

where x = c.c. of thiosulphate solution required by the blank.
y = c.c. of thiosulphate solution required in the test.
w = weight of oil or fat used.

The factor 0·012692 is, of course, for an accurate $\frac{N}{10}$ solution of sodium thiosulphate. If the solution is not exactly $\frac{N}{10}$, the factor must be adjusted accordingly.

The iodine value of English butter ranges from 37–39, French butters from 31–40, and Dutch butters from 31–50. The iodine value of a margarine fat affords a useful clue to the presence of liquid oils, such as cottonseed oil. A typical all-vegetable margarine containing much coconut oil or palm kernel oil would give a low iodine value.

Saponification Value.—The saponification value is the number of milligrams of potassium hydroxide which are required to completely saponify 1 gram of the substance.

[1] *Berichte*, **31**, 750 (1898). [2] *Chem. Zeit.*, **38**, 1111 (1914).

The saponification value increases with decrease in the molecular weight of the glycerides of a fat. Before determining the value for a given oil or fat, the free fatty acid and the unsaponifiable content should be ascertained; the former raises the value, the latter lowers it.

The method was devised by Koettstorfer [1] for butter analysis, and is still conducted on lines very similar to those originally employed: about 2 grams of the clear, filtered oil or melted fat are accurately weighed into a 200 c.cs. flask. Exactly 25 c.cs. of alcoholic potash (approximately $\frac{N}{2}$) are then added, and a reflux condenser is fitted to the flask. A blank test omitting fat is simultaneously conducted. Gentle boiling of the alcohol over a water-bath is maintained until all the fat globules disappear. Thirty minutes boiling is usually quite ample, and saponification is hastened by frequent shaking of the flask. After adding 20 drops of 1% alcoholic solution of phenolphthalein, the test and the blank solutions are titrated whilst hot with accurately standardized $\frac{N}{2}$ hydrochloric acid. The saponification value is calculated from the following formula—

$$\text{saponification value} = \frac{(x - y) \times 0{\cdot}02805 \times 1000}{w}$$

where x = c.c. of acid required in the blank experiment.
y = c.c. of acid required to neutralize the excess of alkali in the actual test.

Most oils and fats yield saponification values lying between 190 and 200. Higher values are given by butter (221–230), coconut oil (254–262), palm kernel oil (244–248) and several fats not met with in margarine practice. Thus the saponification value is a most useful guide in margarine analysis, values above 200 pointing to the possible presence of butter-fat or fats of the coconut group.

Reichert-Meissl Value.—"The Reichert-Meissl value indicates the number of c.c. of $\frac{N}{10}$ alkali required to neutralize the water-soluble volatile fatty acids obtained from 5 grams of an oil or fat which has been saponified, acidified, and distilled in steam under certain arbitrary conditions."

Hehner in 1872 concluded that of the fatty acids obtained from

[1] *Zeits. anal. Chem.*, **18**, 199 (1879).

butter-fat, 5% at least were volatile. He also showed that the majority of other oils and fats gave a much larger yield of insoluble fatty acids than did butter-fat. The first systematic attempts at butter analysis were due to Angell and Hehner.[1] They subjected butter-fat to steam distillation, seeking to determine the amount of volatile fatty acids thus produced. Very irregular results were obtained and the method proved unsuccessful. The pioneer work on our present method of distinguishing between the volatile and non-volatile fatty acids produced when butter-fat was saponified (with NaOH) and then acidified (with tartaric acid) and distilled, was done by Lechartier.[2] The distillate containing the soluble volatile fatty acids was neutralized with baryta, and the barium salts weighed. Under the conditions of Lechartier's experiments, 50 grams of butter-fat yielded about 6 grams of barium salts, whereas tallow gave about 0·3 gram.

Jensen[3] has shown that the distillate (R.M.) of butter-fat contains 85–88% of total butyric acid, 85–100% of total caproic acid, and 24–25% of total caprylic acid, so that it is absolutely essential to apply standard conditions of working. The estimation of the TOTAL volatile fatty acids is impracticable, because of the non-volatile acids decomposing with the formation of volatile acids. Consequently Reichert[4] devised an *arbitrary* or *standard* process, whereby a given *proportion* of the volatile fatty acids could be estimated. His original proposal to work on 2·5 grams of fat is now rejected in favour of Meissl's[5] suggestion to use 5 grams. In an arbitrary method of this nature it is most important that the same experimental conditions should be consistently observed. Briefly, the method consists in saponification of the oil or fat, followed by solution of the soap in water; addition of sulphuric acid liberates the fatty acids, which are then distilled off. The distillate is filtered and titrated with $\frac{N}{10}$ alkali.

The original Reichert-Meissl estimation was carried out as follows: 5 grams of fat are weighed into a 200 c.cs. flask and saponified with 2 grams KOH in 50 c.cs. of 70% alcohol, and the mixture heated on the water-bath until all the alcohol had evaporated. The soap thus obtained is dissolved in 100 c.cs. of water, and 40 c.cs. of dilute sulphuric acid are added, together

[1] *Butter: Its Analysis and Adulteration* (London, 1874).
[2] *Annales agronomiques* (1875), p. 456.
[3] *Zeit. Nahr. Genussm.* (1905), p. 272.
[4] *Zeit. anal. Chem.*, **18**, 68 (1879).
[5] Dingler's *Polyt.*, **233**,222.

with a little pumice. The solution is now distilled and 110 c.c. collected within sixty minutes. This distillate is filtered and 100 c.cs. titrated with $\frac{N}{10}$ KOH, using litmus as indicator. The number of c.cs. of $\frac{N}{10}$ KOH required, multiplied by 1·1, expresses the Reichert-Meissl value of the fat examined.

In 1887, Wollny [1] published his well-known paper on the Reichert-Meissl process, and raised several objections to the test—

He pointed out that—

(*a*) During saponification carbon dioxide may be absorbed to such an extent that an error of + 10% might ensue.

(*b*) Esterification may accompany saponification and lead to losses of 8%.

(*c*) Esterification may occur during the distillation and lead to losses of 5%.

(*d*) Owing to the cohesion of the fatty acids during distillation losses as great as 30% may be entailed.

(*e*) The shape and dimensions of the apparatus employed, and the time employed in distilling, may influence the RM. figure to the extent of ± 5%.

According to Lewkowitsch,[2] these objections have been refuted.

Wollny's modification of the estimation was investigated by a joint committee of the Government Laboratory and the Society of Public Analysts, and the working as prescribed in their report must be rigidly followed. The apparatus used must conform to the dimensions given in the accompanying diagram (see fig. 17). The estimation is carried out as follows—

" Five grams of the liquid fat are introduced into a 300 c.cs. flask, of the form seen in the figure (length of neck 7 to 8 c.mm., width of neck 2 c.mm.). Two c.cs. of a solution of caustic soda, (98%) in an equal weight of water—preserved from the action of atmospheric carbonic acid—and 10 c.cs. of alcohol (about 92%) are added, and the mixture is heated under a reflux condenser connected with the flask by a T-piece, for fifteen minutes, in a bath containing boiling water. The alcohol is distilled off by heating the flask on the water-bath for about half an hour, or until the soap is dry. One hundred c.cs. of boiling water, which has been kept boiling at least ten minutes, are added, and the

[1] *Analyst*, **12**, 203 (1887).
[2] *Oils, Fats and Waxes* (London, 1913), Vol. II. p. 418.

flask heated until the soap is dissolved. Forty c.cs. of normal sulphuric acid and three or four fragments of pumice or broken pipe-stems are added, and the flask is at once connected with a condenser by means of a glass tube 7 mm. wide and 15 c.mm. from the top of the cork to the bend. At a distance of 5 c.mm. above the cork is a bulb 5 c.mm. in diameter. The flask is supported on a circular piece of asbestos, having a hole in the centre 5 c.mm. in diameter, and is first heated by a very small flame, to fuse the insoluble fatty acids, but the heat must not be sufficient to cause the liquid to boil. The heat is increased; and when fusion is complete, 110 c.cs. are distilled off into a

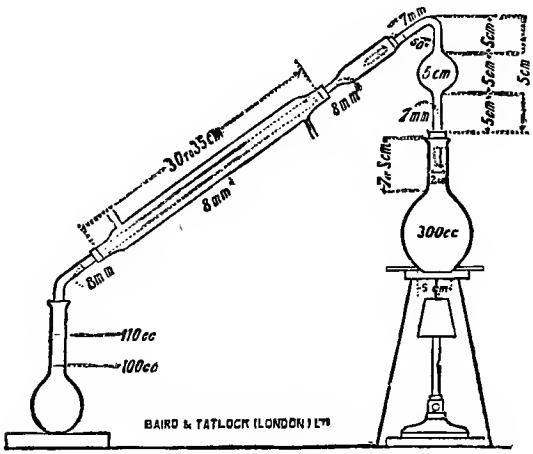

FIG. 17.

graduated flask, the distillation lasting about thirty minutes (say from twenty-eight to thirty minutes), the distillate is shaken, 100 c.cs. filtered off, transferred to a beaker, 0·5 c.cs. of phenolphthalein solution (1 gram in 100 c.c.s) of alcohol added, and the filtrate titrated with decinormal soda or baryta solution. Precisely the same procedure (with the same reagents), omitting the fat, should be followed, and the amount of decinormal alkali required to neutralize the distillate ascertained. This should not exceed 0·3 c.c. The volume of decinormal solution of alkali used, less the figure obtained by blank experiment, is multiplied by 1·1. The number so obtained is the 'Reichert-Wollny Number.'

"*Notes on the Method.*—The sample is melted and filtered from

curd and water through a dry filter. From the filtrate the 5 grams of fat for the process are taken. The soda solution is filtered clear from carbonate formed in its preparation and kept in a special bottle. The Soxhlet spherical condenser is a convenient one for the reflux distillation. This is fixed near the water-bath in which the saponification is to take place, and is connected with the flask by means of a T-piece and indiarubber tubes inclined at an angle of 45°. During the saponification, the free limb of the T-piece is directed upwards, and its end closed by a short piece of indiarubber and glass rod. At the end of fifteen minutes this end is turned downward, and the piece of glass rod replaced by a tube carrying away the alcohol.

"One hundred c.cs. of hot distilled water are added and the flask frequently shaken until the soap is dissolved. The Liebig is a convenient form of condenser. One containing a column of water 30–35 c.cs. in length gives sufficient condensing surface. After shaking the distillate, about 5 c.cs. are filtered through a dry filter into a 100 c.cs. flask. This serves to wash out the flask. When the 100 c.cs. are transferred to a beaker, the flask is not washed out, but the main quantity is neutralized with the standard solution of alkali, and returned to the flask, then again transferred to the beaker, and the titration completed." [1]

The present method of carrying out the test embodies a suggestion of Leffmann and Beam,[2] that instead of saponifying with alcoholic potash, a solution of caustic soda in glycerol is used. This obviates esterification of the volatile fatty acids, and, moreover, it is very convenient and rapid. The method is conducted as follows—

Five grams of the filtered melted fat are weighed into a 300 c.cs. flask, and 20 c.cs. of glycerol-soda (50 grams NaOH dissolved in 50 c.cs. water; 50 c.cs. of this solution are mixed with 450 c.cs. of glycerol). Cautiously heat over a free flame with constant agitation until the mixture suddenly clarifies (two to three minutes). Cool to below 100° C. and add 100 c.cs. of recently boiled distilled water, shaking until the soap is dissolved. Now add 40 c.cs. of normal sulphuric acid and about 0·1 gram of powdered pumice, and immediately connect to the standard condenser and distil off 110 c.cs. into a graduated 110 c.cs. flask in about thirty minutes. Mix the distillate well, and filter 100 c.cs. After adding 0·5 c.c. of 1% alcoholic

[1] *Analyst*, **25**, 309 (1900).
[2] *Ibid.*, **16**, 153 (1891). See also Karsch., *Chem. Zeit.* (1896), p. 207.

phenolphthalein solution, titrate with $\frac{N}{10}$ NaOH until a permanent faint pink colour ensues. The number of c.cs. of $\frac{N}{10}$ alkali, multiplied by 1·1, furnishes the Reichert-Meissl number. A blank experiment is conducted, omitting the fat, but not more than 0·3 c.c. of $\frac{N}{10}$ alkali should be required to neutralize the distillate.

Butter-fat, since it contains glycerides of butyric and caproic acids, has a very high Reichert-Meissl value, viz. 26–33 (average 28). Coconut oil with values from 6–8, cohune oil about 8, and palm kernel oil 5–6, come next in order. The majority of oils and fats have values of about 0·5–1·0. Consequently the test is of great value in butter analysis, for the Reichert-Meissl number would be reduced if the butter were adulterated with lard or oleo. When the adulteration is made with coconut oil or its congeners, the Reichert-Meissl number is not a fair indication, since owing to the variations in the values for different butters, as much as 20% of coconut oil could pass undetected.

Thorpe [1] published results of the determination of the Reichert-Meissl numbers of 357 samples of English butters, and his figures range from 22·5–32·6. The values vary with the time of the year, and with the nature of the feeding-stuffs used for the cattle. The following table, due to Thorpe, shows the variations which may take place in the values of the Reichert-Meissl determination at different seasons—

	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April
Minimum	26·5	26·0	22·5	23·4	22·4	22·3	23·3	23·9	25·6	22·0	27·1	25·3
Maximum	32·8	32·8	31·4	30·5	29·5	29·6	32·9	30·4	31·0	31·6	34·3	33·1

Grimaldi [2] has drawn attention to the fact that the Reichert-Meissl value is influenced by the presence of benzoic acid in a butter or margarine, this acid sometimes being used as a preservative, especially on the Continent. Since benzoic acid is volatile in steam, the Reichert-Meissl value is increased; consequently, should this preservative be detected in a butter or margarine,

[1] *Journ. Chem. Soc.*, **85**, 254 (1904).
[2] *Ann. Lab. Chim. Gabelle*, **6**, 631 (1912). Cf. also Bemelmans, *Zeits. Nahr. Genussm.*, **13**, 492 (1907).

the melted fat must be well agitated with a 5% solution of $NaHCO_3$ to effect its removal.

The present author has examined a sample of butter whose Reichert-Meissl value was 33·39. With 1% of sodium benzoate present, the value had increased to 34·23. Bolton and Revis [1] have determined the effect of the addition of benzoic acid to margarine, and obtained the following results—

Sample.	Reichert-Meissl value.
Margarine mixture	5·87
,, ,, + 0·5 % benzoic acid . .	6·48
,, ,, after bicarbonate treatment .	5·85
Margarine mixture + 2 % benzoic acid . . .	7·59
,, ,, after bicarbonate treatment .	6·03

Polenské Value.—An extension of the Reichert-Meissl method was proposed by Salkowski,[2] namely, to determine the *insoluble* volatile fatty acids, as well as the soluble. Such estimation was put into a practical form by Polenské,[3] and the test bearing his name is now carried out as follows—

Accurately weigh 5 grams of the melted and filtered fat into a 300 c.cs. flask. Add 2 c.cs. of NaOH solution (NaOH dissolved in an equal weight of water) and 20 grams pure glycerol. Heat carefully over a free flame with constant agitation, until the mixture suddenly becomes quite clear. This clarification is very characteristic. Cool to below 100° C. Now add 100 c.cs. of distilled water and effect solution of the soap, by warming if necessary. The pale straw-coloured solution should be quite clear. Acidify with 40 c.cs. of sulphuric acid (25 c.cs. of the pure concentrated acid made up to 1 litre with distilled water; 35 c.cs. should just neutralize 2 c.cs. of the caustic soda solution). After adding 0·1 gram of powdered pumice passing " 90 mesh," connect the flask to the standard condenser, and carefully raise to the boiling-point. The same standard apparatus as used for the Reichert-Meissl determination is again employed, and even more insistence must be placed on the necessity for strictly following the instructions. Using a naked flame, distil off 110 c.cs. in nineteen to twenty-one minutes, into a graduated 110 c.cs. flask.

[1] *Fatty Foods* (London), p. 281.
[2] *Zeit. anal. Chem.*, **26**, 581 (1887).
[3] *Arbeit. aus dem kaiserl. Gesundheitsamte* (1904), p. 545.

The temperature of the distillate should not exceed 21° C. As soon as 110 c.cs. are collected, remove the flame and replace the flask with a 20 c.cs. measuring cylinder. The flask is now immersed up to the 110 mark in water at 15° C. for about five minutes. The oily drops of floating acids are made to adhere to the sides of the flask by carefully tapping, and the flask again cooled at 15° C. for another ten minutes. The physical state of the insoluble acids is noted (oily drops, semi-solid, or solid), since useful information is thus obtained (see p. 110); the flask is now closed with a cork, and the contents well mixed prior to filtering off 100 c.cs. through a filter-paper 8 c.cs. in diameter. The filtrate is titrated with $\frac{N}{10}$ KOH or baryta, as in the Reichert-Meissl determination. The insoluble fatty acids on the paper are washed three times with successive portions of 15 c.cs. of distilled water, each of which has also been used to wash the condenser tube, the 20 c.cs. cylinder, and the 110 c.cs. flask; these washings are neglected. By washing the condenser tube, the cylinder and the flask with three successive portions of 90% alcohol (15 c.cs. each time), the insoluble fatty acids are brought into solution. The alcoholic washings are poured over the filter paper, each portion of alcohol being allowed to drain through before the next is used. The alcoholic filtrate is titrated with $\frac{N}{10}$ alkali, using 0·5 c.c. of 1% phenolphthalein solution (in alcohol) as indicator. The result (less a blank determination) gives the Polenské figure of the oil or fat examined.

The Polenské figure, or "New Butter Value," as it is sometimes termed, is dependent on the presence in a fat of the glycerides of caprylic, capric and lauric acids, as well as, to a lesser degree, of myristic and palmitic acids. Hence, coconut oil and other oils of the same group, containing these glycerides as an essential part of their composition, exhibit high Polenské figures, whilst butter-fat yields a low value. Thus—

Sample.	Polenské figure.
Butter-fat	1·6–3·5
Coconut oil	15–18
Cohune oil	15–16
Palm kernel oil	9–12
Most other oils and fats	about 0·5

In the case of butter-fat, the Polenské figure varies with the

Reichert-Meissl value; this is shown in the following table, due to Polenské—

Reichert-Meissl value.	Polenské figure.	Reichert-Meissl value.	Polenské figure.
19·9	1·35	26·2	1·9
21·1	1·4	26·5	1·9
22·5	1·5	26·6	1·8
23·3	1·6	26·7	2·0
23·4	1·5	26·8	2·0
23·6	1·7	26·9	2·1
24·5	1·6	26·9	1·9
24·7	1·7	27·5	1·9
24·8	1·7	27·8	2·2
24·8	1·6	28·2	2·3
25·0	1·8	28·4	2·3
25·1	1·6	28·8	2·2
25·2	1·6	28·8	2·5
25·3	1·8	29·4	2·6
25·4	1·9	29·6	2·8
25·6	1·7	29·5	2·5
25·4	1·7	30·1	3·0

Richmond [1] has shown from the results obtained in over 100 experiments, that the relationship between the Reichert-Meissl and Polenské values of butter-fat is approximately given by the formula—

$$0{\cdot}033\ R - 0{\cdot}6155 = \log. \frac{10}{\Delta}(P - 0{\cdot}48)$$

where R and P are the respective Reichert-Meissl and Polenské results.

Coconut oil or palm kernel oil is indicated when the Polenské figure is greater by at least 0·5 c.c. than that deduced from the table with reference to the Reichert-Meissl number as given by experiment. An approximate estimation of the amount of coconut oil in a butter is obtained by substituting in the formula—

$$C = \frac{P - P'}{14{\cdot}4} \times 100$$

where C = % coconut oil.
P = found Polenské figure.
P′ = mean Polenské figure from the table, for a value equal to the Reichert-Meissl number + half the value of P.

[1] *Analyst*, **44**, 166 (1919).

A formula more closely in agreement with experimental results is due to Sundberg [1]—

$$C = \frac{1000(P_1 - P_2)}{100 + 10(P_1 - P_2)}$$

where C = % coconut oil.
P_1 = Polenské figure found by experiment.
P_2 = Polenské figure corresponding to Reichert-Meissl value of the mixture.

The Kirschner Process.—Jensen [2] suggested that an estimation of the amount of caprylic acid in the Reichert-Meissl distillate would indicate the presence of coconut oil in a butter, since caprylic acid occurs to far greater excess in coconut oil than in butter-fat. The Reichert-Meissl distillate of butter-fat is rich in butyric acid, and since silver butyrate is easily soluble in water, whereas silver caprylate is only sparingly soluble, the relative amounts of the volatile fatty acids may thus be quantitatively ascertained. Kirschner [3] worked out a process based on this suggestion, and the method, bearing his name, is carried out as follows—

The Reichert-Meissl distillate is neutralized with $\frac{N}{10}$ baryta, silver sulphate added, the liquid filtered, and the filtrate made acid with sulphuric acid, and then distilled as in the Polenské determination. The result indicates the amount of butyric acid present and thus the amount of butter-fat. The process is of great value in the analysis of a margarine which may contain animal fats together with coconut and palm kernel oil, and butter-fat also. From the Reichert-Meissl and Polenské values only, the presence of butter-fat might not be detected, whereas the Kirschner value would definitely indicate its presence or absence. Quantitatively the test is conducted thus—

A hundred c.cs. of the Reichert-Meissl distillate is very accurately neutralized with $\frac{N}{10}$ baryta, and 0·1 gram of silver sulphate added in fine powder form. The mixture is allowed to stand about an hour with frequent shaking. The liquid is then filtered, 100 c.cs. of the filtrate placed in a 300 c.cs. flask, 35 c.cs. of water and 10 c.cs. of sulphuric acid added (25 c.cs. acid in 1 litre).

[1] *Zeits. Nahr. Genussm.*, **26**, 422 (1914).
[2] *Farmaceutisk Titende* (1903), p. 385.
[3] *Zeits. Nahr. Genussm.*, **9**, 65 (1905).

A stout piece of aluminium wire is added to obviate bumping. 110 c.cs. are distilled off, using the standard Polenské apparatus, in about twenty minutes. 100 c.cs. of the distillate are titrated with $\frac{N}{10}$ NaOH. After correcting for a blank determination, the number of c.cs. used is calculated to the Kirschner value by the following formula—

$$K = A \times \frac{121\ (100 + B)}{10{,}000}$$

where A = the corrected Kirschner titration,

and B = the number of c.cs. of $\frac{N}{10}$ baryta used to neutralize 100 c.cs. of the original Reichert-Meissl distillate.

Butter-fat gives values ranging from 19 to 26; coconut oil averages 1·9; palm kernel oil 1·0; and the majority of other oils and fats about 0·1 to 0·2.

The percentage of butter-fat in a margarine is calculated from the following formula, which gives good results—

$$\%\ \text{butter-fat} = \frac{\text{Kirschner value} - (0{\cdot}1\ \text{Polenské value} + 0{\cdot}24)}{0{\cdot}244}.$$

It has been shown by Bolton, Richmond and Revis,[1] and also by Cranfield,[2] that a definite relationship exists between the Kirschner (K) and Polenské (P) values of butter. Richmond[3] gives the following formula as yielding results well in accordance with the mean figures—

$$P = 0{\cdot}26\ (K - 14).$$

Barium Method of Avé-Lallement.—A process due to Avé-Lallement[4] is on similar lines to that of Kirschner. This process is particularly useful in that it at once indicates the adulteration of butter with lard, oleo or vegetable oils (excluding the coconut group) which might be overlooked in a Reichert-Meissl determination.

The method depends on the relative solubility of the barium salts of the fatty acids obtained from a fat. An arbitrary formula is employed, viz.: $b - (200 + c)$, where b = insoluble baryta number, and c = soluble baryta number. For butter-fat the formula is always *negative*, but rapidly passes to a positive value,

[1] *Analyst*, **37**, 183 (1912).
[2] *Ibid.*, **40**, 439 (1915).
[3] *Ibid.*, **44**, 166 (1919).
[4] *Zeits. Nahr. Genussm.*, **14**, 317 (1907).

when butter is adulterated with other oils and fats, all of which yield positive values. Examples [1]—

	Baryta values.			
	a (total).	*b*.	*c*.	$b - 200 - c$.
Butter-fat. . . .	312·0	253·4	58·6	− 5·2
Coconut oil . . .	351·8	294·5	57·3	+ 37·2
Cohune nut oil . . .	348·3	292·8	55·5	+ 37·3
Palm kernel oil . . .	336·6	303·7	32·9	+ 70·8
Oleo oil	274·5	261·2	13·3	+ 47·9

The Avé-Lallement method is conducted as follows—

Five grams of fat are saponified with 50 c.cs. of $\frac{N}{2}$ alcoholic KOH. The solution is neutralized with $\frac{N}{2}$ HCl, whence the saponification value is calculated. The alcohol is boiled off, the evaporation being assisted by blowing air into the flask, and the soap is dissolved in hot, recently-boiled, distilled water.

The soap solution is made up to exactly 250 c.cs. at 40° C., and 100 c.cs. are pipetted out. This portion is placed in boiling water for about five minutes, and to it are added 50 c.cs. of $\frac{N}{5}$ $BaCl_2$ (25 grams barium chloride in 1 litre), and the mixture retained on the water-bath until coalescence of the precipitated barium salts is complete. Cool. Filter into a 250 c.cs. flask, and make up to the mark with distilled water. 200 c.cs. are pipetted into a beaker, 1 c.c. of strong HCl added, brought to the boiling-point, and 10 c.cs. of approximately normal H_2SO_4 added. The precipitate is allowed to settle overnight, and then filtered on to a Gooch crucible, washed free from chlorides with very dilute H_2SO_4 water, and finally washed with two 10 c.cs. portions of warm alcohol. Dry to constant weight.

Calculation.—The weight of $BaSO_4$ found in 200 c.cs. of the filtrate is increased by 25%, and calculated in terms of milligrams of BaO ($BaSO_4 \times 0{\cdot}657 \times 1000$). This result is deducted from the BaO value (in milligrams) of the 50 c.cs. of $BaCl_2$ solution added. The BaO value of the acids yielding insoluble salts from 2 grams of fat is thus obtained, and this, calculated to 1 gram fat, gives the insoluble BaO value (*b*). The saponification number

[1] From Bolton and Revis, *Fatty Foods* (London).

of the fat is multiplied by 1·368, so as to yield the total BaO value (a). Then ($a - b$) = soluble BaO value = c.

Blichfeldt's Process.—In 1910, Blichfeldt [1] described a method for estimating butter and fats of the coconut group in margarine. The original method has been revised, and a later publication [2] contains the summaries of extensive tests on the process.

The method entails a distillation of fatty acids as in the Reichert-Meissl-Polenské determination, but the soluble and insoluble fatty acids are not separated. By means of a special apparatus (fig. 18) all the volatile acids are collected without loss, dissolved in standard NaOH, and the excess of alkali titrated with standard acid. The insoluble silver salts of the volatile fatty acids are now precipitated by adding a known volume of $\frac{N}{10}$ silver nitrate, and the precipitation hastened by addition of pure sodium nitrate. The excess of silver nitrate is determined by a suitable titration, *e. g.* with $\frac{N}{10}$ sodium chloride, and thus the difference between the equivalent of the total volatile fatty acids and the equivalent of the acids forming insoluble silver salts, yields the equivalent of the soluble silver salts.

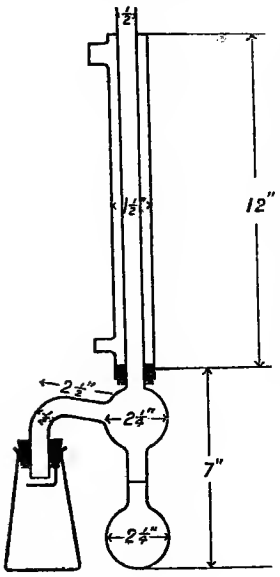

FIG. 18.

The special reagents required in Blichfeldt's method are—

1. Caustic potash solution, made by dissolving potassium hydroxide in an equal weight of distilled water.

2. A solution of sulphuric acid containing 12·5 grams of concentrated acid per litre.

3. Iron alum indicator, made by adding to a saturated solution of iron alum half its volume of nitric acid (one part of concentrated acid to three parts of distilled water).

Twenty grams of the filtered fat are accurately weighed into a 300 c.cs. "resistance" Erlenmeyer flask, and 8 c.cs. of the special potash solution and 25 c.cs. of glycerol are added. Cautious

[1] *Journ. Soc. Chem. Ind.*, **29**, 792 (1910). [2] *Ibid.*, **38**, 150, T (1919).

heating over a free flame with constant agitation is maintained until saponification is complete. The pale-yellow product is cooled and then made up to 200 c.cs. with distilled water which has been freed from carbonic acid by boiling.

Fifty c.cs. of this soap solution, corresponding to 5 grams of the fat, are measured into a 300 c.cs. "resistance" conical flask, 100 c.cs. of the special sulphuric acid are added, and 0·1 gram of pumice powder is sifted into it through butter muslin.

The flask is now connected to the standard apparatus and 100 c.cs. distilled over in about twenty minutes. The mark between the two bulbs indicates a volume of 100 c.cs. at 65° C. At the end of the distillation the flask and condenser jacket are removed and the side tube closed by a cork. 0·5 c.c. of a 1% solution of phenolphthalein in alcohol is added, and a known excess of from 5–10 c.cs. of $\frac{N}{10}$ NaOH is now introduced through the condenser tube, which is then closed by a cork. The volatile acids are completely dissolved in the hot alkali solution by shaking; at the beginning of the shaking operation the cork is withdrawn once or twice to reduce the pressure inside. The resulting solution of sodium salts of the volatile acids is transferred to a 200 c.cs. measuring flask, and the condenser tube is rinsed out several times with warm water into the flask. The volatile acids are then determined in the cooled liquid by difference by titrating the excess of alkali with $\frac{N}{10}$ sulphuric acid, 0·4 c.c. for a blank experiment being deducted.

The insoluble silver salts are now precipitated from this neutral liquid by adding $\frac{N}{10}$ silver nitrate (5 c.cs. in excess of the number of cubic centimeters of $\frac{N}{10}$ NaOH required to neutralize the volatile acids), and complete precipitation of the insoluble silver salts from the solution is effected by dissolving in it 20 grams of pure sodium nitrate. The liquid is then made up to 200 c.cs., repeatedly shaken for about five minutes, and 175 c.cs. filtered into a measuring flask. The filtrate is transferred to a 300 c.cs. flask, and, after adding 15 c.cs. of the iron alum indicator, titrated with $\frac{N}{10}$ potassium thiocyanate until a red coloration just appears. The number of cubic centimeters of thiocyanate solution required $\times \frac{8}{7}$, subtracted from the number of cubic centimeters of silver

nitrate used, gives the equivalent of the insoluble silver salts; the latter, subtracted from the figure representing the total volatile acids, gives the equivalent of the soluble silver salts.

The following results, averages of numerous determinations on widely different samples, are given by Blichfeldt—

Sample.	Total.	Insoluble.	Soluble.	Melting-point of insoluble volatile acids.
Coconut oil . . .	21·0	17·6	3·4	8–12° C.
Palm kernel oil . .	13·7	11·0	2·7	21–24° C.
Butter-fat . . .	29·6	5·0	24·6	—
Lard	—	0·4	—	—
Arachis oil . . .	—	0·5	—	—

The melting-point of the insoluble volatile acids of coconut oil varies from 8–12° C., and that of palm kernel oil from 21–24° C. For mixtures of these fats, the melting-point of these acids coincides with the value given by direct calculation. This is a valuable aid in margarine analysis. The insoluble volatile acids may be obtained by making a second distillation and simply using the oil floating on the top of the distillate, or they can be liberated from the precipitated silver salts remaining on the filter in the course of the general method. The silver salts are transferred to a narrow glass cylinder, the acids liberated by adding two drops of HCl, and then removed in ethereal solution by extracting twice with 10 c.cs. of ether.

The Blichfeldt method is a good check on the results obtained by the Reichert-Meissl-Polenské method, and is particularly useful in the analysis of margarines containing both coconut oil and palm kernel oil. It serves, too, for the ready detection of butter-fat, and is thus comparable with the Kirschner determination.

Shrewsbury-Knapp Method.—The characteristic insoluble volatile fatty acids of the fats of the coconut group are lauric and myristic acids, and since these are soluble in alcohol of a definite strength, Shrewsbury and Knapp [1] proposed a method based on these facts for detecting coconut fats in margarines. As modified by Elsdon [2] the process is conducted as follows—

After the usual Reichert-Meissl distillation has been made, the

[1] *Analyst*, **35**, 385 (1910). [2] *Ibid.*, **42**, 298 (1917).

flask containing the residual fatty acids is cooled in water until the acids form a solid cake. This cake is then broken up with a stirring rod, and placed on a fine wire sieve. The flask and fatty acids are washed with 50 c.cs. of cold water. After draining, the fatty acid particles are returned to the flask by means of an iron spatula. The acids are dried in the water-oven, air being blown into the flask at intervals to assist drying. To the flask are now added 100 c.cs. of alcohol, which must be accurately adjusted to exactly 0·9200 S.G. at 15·5° C. The flask is corked and gently warmed until the fatty acids are entirely dissolved. Cool until the temperature is exactly 15·5° C., or, preferably, maintain in a thermostat at 15·5° C. for twenty minutes. The contents are now filtered, and 50 c.cs. of the filtrate titrated with $\frac{N}{10}$ NaOH, using 1 c.c. of 0·2% phenolphthalein solution as indicator. The number of cubic centimeters of alkali required depends on the amount of coconut or palm kernel oil in the original sample. For pure coconut oil, 89·8 c.cs. of $\frac{N}{10}$ NaOH are necessary for the titration.

Elsdon furnishes the following figures, obtained from butter and margarine mixtures—

Per cent. coconut oil.	0.	10.	20.	30.	40.	50.	60.	70.	80.	90.	100.
With butter-fat . .	12·7	14·5	16·5	20·6	24·0	30·4	—	44·4	58·3	76·5	89·8
With oleomargarine .	8·9	10·6	13·7	16·9	20·8	25·4	31·8	41·8	53·4	73·2	89·8

When a margarine contains both coconut oil and palm kernel oil, the presence of the latter is indicated, according to Elsdon, when the Polenské figure, calculated to coconut oil, shows a lower percentage than that calculated from the Shrewsbury-Knapp figure. This statement is based on the fact that coconut oil is richer in the lower fatty acids than palm kernel oil, as indicated by the higher Polenské value of the former, whilst the proportion of lauric and myristic acids is practically the same in coconut and palm kernel oils.

As examples where the Elsdon modification indicates the presence of both coconut oil and palm kernel oil in a margarine, the following may be cited: numbers 1, 2, 4, 6, 8.

Sample.	Reichert.	Polenské.	S. K. (Elsdon's method).	Per cent. coconut oil.		Difference.
				From P.	From S. K.	
1	6·0	8·2	76·0	52	88	36
2	8·3	6·3	29·0	40	50	10
3	3·2	2·5	11·5	21	18	3
4	12·6	9·3	43·9	54	68	14
5	3·8	2·3	12·5	19	20	1
6	5·2	5·0	27·9	34	46	12
7	5·0	4·7	16·9	33	31	2
8	5·4	6·9	37·5	45	61	16
9	2·4	1·3	8·2	11	10	1
10	5·1	4·7	17·3	32	32	0
11	4·6	3·2	14·1	24	24	0
12	5·4	6·1	22·7	40	41	1

Burnett and Revis Method.—This method[1] determines the turbidity-temperature of the alcoholic solution of the barium salts of the volatile insoluble fatty acids. After the Polenské figure has been ascertained, using $\frac{N}{10}$ baryta for the titration, the insoluble barium salts are filtered off, using a hardened filter-paper and the pump. The precipitate is washed with 3 c.cs. of 93% alcohol, and then dissolved in ten times the Polenské value in cubic centimeters of 93% alcohol (sp. gr. 0·8235 at 15·5° C.), using a reflux condenser. The hot solution is transferred to a test-tube enclosed in a boiling-tube, and a small-bulb thermometer inserted. The temperature at which turbidity occurs is then read off.

Coconut oil leads to a turbidity-temperature of 52·5° C., and palm kernel oil, 68·5° C. Since the turbidity-temperature is directly influenced by the relative percentages of coconut and palm kernel oils present, the method is useful in margarine analysis as affording a clue to the difficult problem of estimating the percentages of coconut and palm kernel oils when both are present in the fatty mixture. Confirmation of the result arrived at by this method would be given by a determination of the melting-point of the insoluble volatile acids as in Blichfeldt's process.

The Interpretation of Analytical Results.—Margarines can now be made of such a variety of oils and fats, that a complete quantitative analysis has become a difficult matter, and requires extensive experience in margarine practice. Sometimes a definite composition can be safely assigned; in some cases only generalized statements of fairly wide percentage limits can be given.

[1] *Analyst*, **38**, 255 (1913).

When undertaking the analysis of a margarine, the analyst needs to make as many determinations as possible in various directions, and to interpret his results with constant reference from one analytical value to another. Physical determinations should include melting-point, setting point, refractive index (usually at 40° C.), and microscope tests for beef-fats or lard (Belfield test). Colour reactions for liquid oils are also usual, *e. g.* Halphèn test for cottonseed oil; Milliau-Becchi test for kapok oil; Baudouin test for sesamé oil. Then follow the tests for detecting and estimating coconut and kindred fats, as well as butter-fat, viz.: the Reichert-Meissl-Polenské determinations, with the additional and more detailed information given by the Kirschner, Shrewsbury-Knapp, Burnett-Revis, and Blichfeldt methods. The iodine number and saponification value are also exceedingly important determinations.

The saponification value will indicate the presence of oils of the coconut group, and possibly butter-fat. A value above 200 suggests one or more of these fats. The Reichert-Meissl-Polenské values will show if such fats are present. The Polenské number is practically independent of the butter-fat (if present), and indicates the percentage of coconut and (or) palm kernel oil. A high Reichert-Meissl value and a low Polenské value will together suggest butter-fat, the actual presence of which would be at once definitely determined by the Kirschner value. The values of the Reichert-Meissl, Polenské, and Kirschner results are not appreciably influenced by the other animal or vegetable constituents.

Elsdon's modification of the Shrewsbury-Knapp process will aid in fixing the percentage of coconut oil present, at the same time indicating the presence or absence of palm kernel oil. If the latter be present, the method of Burnett and Revis will aid in deciding the actual percentage. The Blichfeldt process is useful in confirming the results obtained, and the melting-point of the insoluble fatty acids will effectively check the relative percentages of coconut and palm kernel oils as given by the other methods.

It now remains to settle the composition of the remaining constituents. The microscope will reveal lard and (or) beef-fats. The refractive index will show the presence of liquid vegetable oils, colour tests for which are made. (In this connection it is noted that most continental margarines contain sesamé oil. In conducting the Baudouin test, a blank test is also made, owing to certain colouring matters giving a red colour with hydrochloric acid.) The iodine value and the melting-point will suffice to

furnish a fairly definite conclusion of the amounts of animal fats and liquid oils present. If the exact nature of the vegetable liquid oils is not satisfactorily ascertained, the results should be reported as "liquid vegetable oils," and no surmise should be made to indicate them more precisely.

When a hardened oil is suspected, as in some pastry margarines, or lard-compounds, a nickel test should be conducted. This test, however, is not always responded to by hydrogenated oils, and only long experience of margarine analysis can help to decide on the presence of a hardened product.

The following table indicates the range of values resulting from the analysis of various mixtures of oils and fats used in making margarine. By applying the above remarks to the table, the fixing of the final composition can be readily followed—

Analytical value.	A.	B.	C.	D.	E.	F.	G.	H.
Reichert-Meissl No.	2·7	5·9	5·6	1·5	4·2	8·9	7·4	<1·0
Polenské No. .	4·6	4·8	12·0	3·2	9·1	11·4	9·3	<1·0
Kirschner No. .	0·8	2·74	1·5	0·5	0·8	3·7	3·7	—
Total Blichfeldt No.	5·25	8·2	14·6	.5·2	10·0	17·7	15·1	—
Soluble ,, ,,	0·85	3·2	2·2	0·8	1·86	4·9	4·6	—
Insoluble ,, ,,	4·4	4·9	12·4	4·4	8·14	12·8	10·5	—
Saponification value	212·0	214·3	242·7	210	234	242	234	198
Iodine value .	47·71	42·17	29·1	56·1	34·1	32·2	34·0	55·6
Refractive index at 40° C.	46·0	46·0	40·0	46·8	43·0	40·0	40·8	49·3
Melting-point .	27·3	28·8	20·7	23·6	24·8	20·5	21·8	28·0
Halphen test .	++	+	—	+++	+++	+++	+++	+
Belfield test beef	+++	+++	—	+++	+++	—	—	+++
Belfield test lard	—	—	—	+	—	—	—	++
Nickel test . .	+	+	—	—	—	—	—	—

The compositions of the various mixtures as actually compounded in the laboratory for trial purposes are given in the subjoined table—

Fatty constituents.	A.	B.	C.	D.	E.	F.	G.	H.
Butter-fat . .	—	10%	—	—	—	10%	10%	—
Oleo . . .	55	50	—	30	—	—	—	60
Premier Jus . .	—	—	—	10	15	—	—	—
Coconut oil . .	25	25	50	25	15	70	35	—
Palm kernel oil .	—	—	30	—	50	—	35	—
Cottonseed oil .	10	5	—	20	20	20	20	10
Ground-nut oil .	—	—	20	—	—	—	—	—
Lard . . .	—	—	—	15	—	—	—	30
Hardened whale oil	10	10	—	—	—	—	—	—

CHAPTER XI

DETERIORATION OF BUTTER AND MARGARINE IN STORAGE

BOTH butter and margarines undergo more or less rapid changes with time, these changes becoming more quickly apparent when the product is not cold-stored. Much work has been published on rancidity and the development of moulds in butter, but fewer observations of the kind have been made in the case of margarines. The two cases, however, are practically identical, and, in the main, conclusions drawn from investigations in this connection on butter may safely be applied to margarines under the same conditions; particularly is this the case with oleomargarines.

Butter.—The keeping qualities of butter are intimately bound up with the treatment of the cream prior to churning. Raw or fresh cream produces a "sweet cream butter" which does not keep well. Similar butter from cream pasteurized previous to churning shows the best keeping qualities of all classes of butter. Sour cream, natural, or artificially soured by inoculation with *B. lactis acidi* after being pasteurized, produces a butter which keeps much better than that made from fresh unpasteurized cream. Hence it is evident that *B. lactis acidi* also plays a part in butter decomposition. For a time it is helpful, since it overgrows other and noxious types of bacteria during the ripening process. The water used in washing butter free from buttermilk must be thoroughly hygienic, as a serious source of contamination may be introduced, very detrimental in subsequent storage.

Butter decomposition may develop along two main lines: (1) chemical, producing an objectionable tallowy odour and taste; and (2) biological, whereby rancidity is induced by both bacteria and moulds. No definite chemical changes can be dogmatically formulated as accompanying the decomposition of butter, just as no definite enumeration of the micro-organisms producing rancidity can be universally applied. Rancidity and increase in the free fatty acid content usually accompany each other, but

quite frequently a low, free fatty acid content may be found in a very rancid sample. Guthrie [1] looks upon rancidity in butter as a butyric acid flavour, but notes that the acid value is not a correct measure of the degree of rancidity attained.[2] Numerous attempts have been made to determine what chemical changes occur, and what particular bacteria and moulds have the predominating influence in rancidity.

Butter on exposure to light is frequently converted into a white mass resembling tallow, with a bitter taste and unpleasant smell. Lidow and Dshors [3] exposed butter in closed vessels to the influence of the light from Auer-burners, the electric light, and the violet light produced by the combustion of carbon-disulphide in nitric oxide. In each case the yellow colour was bleached and the unpleasant tallowiness was produced. They found that the acetyl number increased from 50 to 87, but observed no change in the Reichert-Meissl and the iodine values. Zilva [4] found precisely the same change take place when butter-fat was exposed to ultra-violet rays, and at the same time it was observed that the accessory food-factor, "fat-soluble A," was completely destroyed. The present writer has observed a bleaching of butter-fat, and the accompanying tallowiness, in a sample of melted and filtered butter-fat, kept in the dark in a refrigerator at 2° C. Bleaching was rapid, and the whole change was complete in five weeks; no sign of mould was apparent.

Hunziker and Hosman [5] conclude that oxidation is the influence producing the characteristic tallowiness, and they suggest that glycollic acid is formed from the free glycerol produced by a partial hydrolysis of the butter-fat, and that the glycollic acid combines with the fatty acids, so producing the compounds of unpleasant flavour. In support of this theory they found that the presence of lactose greatly intensifies the results of oxidation, since it leads to excess of glycollic acid. Nestrelajew [6] obtained results which seem to point to an oxidation effect, and he observed that, under the influence of light, the iodine value of butter-fat decreased. An increase occurred in the saponification value, and in the mean molecular weight of the non-volatile acids.

Mjöen,[7] whilst supporting the oxidation idea, recognized that

[1] *Journ. Dairy Sci.*, **1**, 218 (1917).
[2] Cf. V. Klecki, *Zeit. anal. Chem.*, **34**, 633 (1895).
[3] *Chem. Zeit.*, **27**, *Rep.* 253 (1903).
[4] *Biochem. Journ.*, **13**, 164 (1919).
[5] *Journ. Dairy Sci.*, **1**, 320 (1917).
[6] *Milch. Zentralb.*, **6**, 1 (1910).
[7] *Forschungs Ber.*, **4**, 195 (1897)

light was not essential, though it had a distinctly accelerating effect. Oxidation, he found, was accompanied by a decrease in the iodine value and an increase in the free fatty acids. A sample of butter-fat of which the initial iodine number was 32·7 and the acid value of which was 1·2, was exposed to a current of air in sunlight for three days. The iodine value fell to 30·9, and the acid value increased to 10·7. Another sample of the same fat was kept in the dark, and after exposure to a current of air at 60° C. for thirty-five days, the iodine number had dropped to 19·8, the acid value increasing to 10·7. The colour remained yellow for three weeks, and then quite suddenly turned white.

Rancidity due to Micro-organisms.—On dry oils and fats bacteria and enzymes have only a slight action; in the presence of sufficient water they may exert a marked hydrolytic action on the fatty material.[1] In the case of butter there is also the casein to be considered, and the effect of salt. During rancidity changes the fat may be acted upon by certain fat-splitting bacteria, and also the protein matter may be converted into obnoxious products which confer an undesirable odour and flavour to the butter. Orla-Jensen[2] has shown that the micro-organisms ordinarily producing rancidity are *Oidium lactis; Cladosporium butyri; B. fluorescens liq.;* and, at times, *B. prodigiosus.* All these decompose the butter-fat. Volatile acids are produced, first by the bacteria, and later by the combined action of the two mould fungi. Butyric esters are formed, but may be inhibited by means of lactose. Presence of common salt inhibits the formation of volatile fatty acids. Moulds enter from the air, *B. fluorescens liq.* and *B. prodigiosus* from the wash-water.

Laxa[3] found that, in addition to *Oidium lactis* and *B. fluorescens liq.*, rancidity was also produced by *Penicillium* and *Mucor*, and, to a lesser extent, by the yeasts. He observed that lactic acid bacteria and the different varieties of tyrotrix do not influence butter-fat *per se.* His views on the decomposition of the glycerides agree with those of Hanus,[4] who showed that the action causing rancidity in butter affects the glycerides of the non-volatile higher fatty acids much more easily and rapidly than the glycerides of the volatile fatty acids, but that no difference existed between

[1] White, *Journ. Ind. Eng. Chem.*, **11**, 648 (1919). Cf. also Ritzert, *Rancidity in Fats* (Berlin, 1890).
[2] *Centr. Bakt. Parasit.*, **8** (2), 11, 42, 74, 107, 140, 171, 248, 278, 309, 342, 369, 406.
[3] *Arch. Hyg.*, **41**, 119–51.
[4] *Zeit. Nahr. Genussm.*, **3**, 324 (1900).

the rate of decomposition of the glycerides of the saturated and of the unsaturated acids. Another reason why the decomposition does not proceed uniformly in the case of all the glycerides of butter-fat has been given by Laxa as due to the fact that the liberated soluble fatty acids exert a destructive action on the mould fungi, the potency of which increases with rising molecular weight. The volatile fatty acids set free are further decomposed by the mould fungi. In the case of *Penicillium* and *Mucor*, Laxa traced the cause of the glyceride-decomposition to the presence of an enzyme. Combs and Eckles [1] have also drawn attention to the abnormal flavours in butter caused by enzymes secreted by moulds in the cream employed.

Thom and Shaw [2] have identified the various types of moulds in butters, and find that mouldiness falls into three classes—

1. Orange-yellow areas with a submerged growth of mycelium are produced by *Oidium lactis*.

2. Smudged or dirty-green areas, either entirely submerged or with some surface growths, are produced by species of *Alternaria* and *Cladosporium*.

3. Green surface colonies are produced by species of *Penicillium*, or, more rarely, *Aspergillus*, either upon the butter, and causing decomposition, or upon the container and wrappings, and thus injuring the appearance of the samples. Excess of curd favours mould development, as also do wet surfaces, wet wrappings, and high humidity. Thom and Shaw recommend the addition of 2·5–3% of salt as a preservative for butter.

From the chemical standpoint, it is now agreed that free fatty acidity bears no definite relationship to the taste and smell of rancid butter. Reinmann [3] proved the accelerating influences of casein and lactose, and found that light exerts no effect in rancidity changes (biological). Atmospheric oxygen does not cause rancidity of this type, and Reinmann has shown that "sterilized cream-butter" with a free access of sterilized air does not turn rancid.

The work of Browne [4] indicates that with advancement of rancidity there appears in the case of butter-fat—

(*a*) A decided increase in acidity, saponification value, acetyl value and Reichert-Meissl value;

(*b*) A slight increase in the ether value, due to the presence of

[1] *Journ. Dairy Sci.*, **1**, 347 (1917).
[2] *Journ. Agric. Research*, **3**, 301 (1915).
[3] *Centr. Bakt. Parisitenk*, **6** (2), 166, 209.
[4] *Journ. Amer. Chem. Soc.*, **21**, 975 (1899).

the aldehydic bodies formed, and to the decomposition of these into acids by the alcoholic potash;

(*c*) A decrease in the iodine number, and in the percentage of insoluble fatty acids and glycerol;

(*d*) The specific gravity (40/15·5° C.) is raised, *e. g.* fresh butter gave values ranging from 0·9050–0·9102, which increased with rancidity development to 0·9195–0·9252.

The Crismer temperature was lowered by 7–12° C. and, as was to be expected, there was a marked decrease in the calories of combustion of the fat, *e. g.* from 9366 to 9095. This is due to oxidation changes having accompanied progress of rancidity. The following table illustrates the changes in the analytical values due to rancidity. Stockmeier's[1] "degrees of rancidity" have been included, and they indicate the number of cubic centimeters of normal alkali required to neutralize the free fatty acids in 100 grams of fat. Thus, one degree of rancidity = an acid number

Age.	Degrees of rancidity.	Acid No.[2]	Saponification value.	Ether No.	Reichert-Meissl value.	Iodine No.	Per cent. oleic acid.
Fresh .	0·9	0·48	228·1	227·6	15·63	34·95	38·79
1 week .	2·3	1·28	230·3	229·0	15·80	34·55	38·35
1 month	19·4	10·90	241·0	230·1	17·0	28·40	31·52
2 months	51·5	28·84	260·0	231·2	18·75	14·35	15·93
4 ,,	53·5	30·00	262·1	232·1	19·80	11·15	12·38
8 ,,	63·2	35·38	269·3	233·9	21·13	8·55	9·49

of 0·56. A second table, due to Browne, illustrates the changes occurring in the values of the acetyl number, glycerol percentage, and percentage of insoluble acids.

Age.	Degrees of rancidity.	Iodine No.	Saponification value.	Acetyl No.	Per cent. insoluble acids.	Per cent. glycerol.
Fresh . .	0·93	34·92	225·6	3·8	88·46	12·33
3 months .	26·43	22·55	245·3	18·0	81·15	11·67

Nagel[3] has separated numerous compounds from rancid oils and fats, and has identified saturated and unsaturated free fatty acids; lactones and anhydrides of fatty acids; hydroxy fatty

[1] *Vierteljahrsschrift Nahr. u. Genussmittel* (1889), p. 428.
[2] Cf. also Amthor, *Zeit. anal. Chem.*, **38**, 10 (1899).
[3] *Amer. Chem. Journ.*, **23**, 173 (1900).

acids; alcohols (*e. g.* butyl, amyl, capryl); esters of saturated, unsaturated, and hydroxy fatty acids with higher and also sometimes polybasic alcohols; aldehydes, saturated and unsaturated; acetals and terpenes.

Action of Salt.—Salt has long been recognized as possessing a preservative action on butter, though recent investigations show certain limitations exist. Some moulds cannot live in 2% salt solution, *e. g.* species of *Oidium*, *Alternaria*, and *Cladosporium*. Very interesting conclusions concerning the influence of salt in storage butter were made by Washburn and Dahlberg [1] in 1917. They found—

1. Common salt, exclusive of its antiseptic property, hastened the deterioration of butter.

2. When stored at −15° F. (−26° C.) unsalted butter kept just as well as when salted.

3. The bacteria in the unsalted butter *decreased* more rapidly at −15° F. than they did in the salted, but they *increased* more rapidly at + 58° F. (14·4° C.).

4. The acidity of the salted and unsalted butters increased uniformly at −15° F., but at 58° F. the increase was greater in the unsalted samples.

5. At −15° F. only the salted butters lose moisture.

6. Little, if any, relation exists between the bacteria, acidity, and the quality of the butters investigated.

Browne [2] has shown that casein is decomposed by the butter flora in the presence of salt, being slowly broken down into amino-acids and ammonia. A sample of freshly-churned butter was divided into two portions, one of which was then salted. The total nitrogen (in the form of amino-acids and ammonia) was 5·71%. After two hundred and forty days storage at −6° C. the nitrogen figure had increased to 7·59% in the unsalted sample and 8·19% in the salted.

It has often been noticed that when fresh butter is squeezed the exuding liquor is milky, whilst in the case of salted butter the liquor is quite clear. This is due to the salt dissolving in the water (as buttermilk) in the butter, and then coagulating the protein matter, probably as casein lactate. Salt does not change the *colour* of butter-fat at all, and the so-called "mottled butters," exhibiting marked variations in colours as streaks, waves, spots, or blotches, acquire their undesirable appearance owing to (1) the presence in excess, and uneven distribution of, the buttermilk,

[1] *Journ. Dairy Sci.*, **1**, 114 (1917). [2] *Science*, **42**, 319 (1915).

and (2) the precipitated proteid matter caused by the action of brine. No mottling occurs when salt is absent, or when buttermilk is not present in excess; scientific investigations on this mottling effect have been made by van Slyke and Hart,[1] who found a time factor involved.

Cold Storage.—During cold storage butter loses a little moisture by evaporation; other purely physical and chemical changes are reduced to a minimum. In time, cold storage of butter produces a falling-off in flavour, which may be induced by several factors. Any volatile flavours in the room are liable to absorption. Bacteria, moulds, and yeasts may gain ingress from the circulating atmosphere. It is now generally agreed that though cold storage greatly inhibits bacterial growth, it does not destroy it. Complete inhibition is only produced by actually freezing the butter. Certain types of micro-organisms can thrive, even if slowly, a degree or two above 0° C., and any slight changes in the temperature and humidity of the room may accelerate their development. Enzymes need not be considered as a factor in the deterioration of butter in storage, and results in support of this view have been gained by Thatcher and Dahlberg.[2]

A most interesting investigation on the action of cold on micro-organisms has been carried out by Ruata.[3] He found that the micro-organisms commonly attacking food substances have their development suspended at low temperatures, but that they are not destroyed. In a dry atmosphere at temperatures ranging from −3° C. to −12° C. the growth of various common bacteria was investigated. *B. coli* soon showed a retarded development, and the action of the cold was progressively germicidal. In one hundred and fifteen to one hundred and twenty days the culture was completely destroyed. *B. pyocyaneus* also suffered a progressive destruction, and its ability to liquefy gelatin was inhibited by long exposure to low temperature. The liquefying action on gelatin usual with *Staphylococcus pyogenes aureus* was progressively delayed, but not destroyed; similarly with respect to its hæmolytic capacity. After six months cold storage *B. proteus vulgaris* and *B. bulgaricus* were still able to coagulate milk, though the former could no longer liquefy gelatin. Prolonged exposure to low temperature completely inhibited the amylolytic power of *B. clavatus*, Biffi, and its usually remarkably

[1] *Journ. Amer. Chem. Soc.*, **27**, 679 (1905).
[2] *Journ. Agric. Research*, **11**, 437 (1917).
[3] *Bull. Agric. Intell.*, **10**, 116 (1919).

resistant spores were gradually destroyed. Similar results were obtained with *B. coliformis, B. fluorescens non liquefaciens, B. prodigiosus,* and *B. proteus vulgaris* (isolated from putrefying meat).

In the case of butter, it has long been recognized that sweet cream butter deteriorated less than sour cream butter. Blanchet,[1] in his study of "Enzyme Activity at Cold Storage Temperatures" concluded that the keeping quality of butter was inversely proportional to the degree of acidity at the time of churning the cream. Similar observations were made by Rogers and Gray.[2] Rogers and his collaborators[3] found that butter made from sweet pasteurized cream kept much better than butter made from similar but unpasteurized cream, but they were unable to reproduce the changes in the unpasteurized cream by re-inoculating the pasteurized cream with the bacteria of the raw cream. A most interesting observation due to these investigators concerned the *gas* content of butter. Fresh butter furnishes 10% by volume of gas, containing 20% oxygen, 33% nitrogen, and 47% carbon dioxide. The oxygen content materially decreases during storage of the butter.

An important contribution to the subject has been made by Dyer,[4] on the "Progressive Oxidation of Cold Storage Butter." Briefly stated, his conclusions are that the deterioration in flavour at 0° F. is not dependent on an oxidation of the fat itself, but is due to chemical changes of slow oxidation in some of the non-fatty substances of the buttermilk. This change is proportional to the acidity of the cream used in churning the butter. Dyer found that the quantity of carbon dioxide varied directly with the amount of buttermilk retained in the butter, and that during storage the carbon dioxide increased to a maximum percentage, and then progressively decreased.

Margarine.—In 1902 Crampton[5] examined fifty samples of the same parcel of oleomargarine, which were kept in stoppered bottles for two years. From his experiments he concluded that in the case of edible fats, where the presence of nitrogenous and other non-fatty matter affords a nutritive medium for such growth, the greater part of the changes embraced under the general term "rancidity" are due to the action of micro-organisms or to enzymes

[1] *Le Froid,* **5,** 6 (1917).
[2] U.S. Dept. Agric., Bur. An. Ind., *Bull.,* **114.**
[3] Loc. cit., *Bull.,* **162,** 5–69.
[4] *Journ. Agric. Research,* **6,** 927 (1916).
[5] *Journ. Amer. Chem. Soc.,* **24,** 711 (1902).

resulting from their growth. The following table of average analytical figures was obtained—

Group.	Condition.	Reichert-Meissl No.	Refractive index (25° C.).	Specific gravity $\frac{40}{40°}$ C.	Valenta value.	Melting-point °C.	Iodine No.
(A) 19 samples	No mould	4·77	1·4646	0·9084	77·0	45	44·28
(B) 20 ,,	Slight mould	3·82	1·4633	0·9080	36·5	45	43·66
(C) 9 ,,	Very mouldy	2·98	1·4610	0·8979	31·0	45	49·81

The original Reichert-Meissl value was 2·42. The refractive index and Valenta value approach the figures for butter in the case of the heavily-moulded samples. The mould was evident as black spots, entirely penetrating the mass. In each case the bulk of the black substance was *Coniothecium*, and in a few samples only was *Aspergillus* detected. Crampton has collected the results of Hanus and Stockey, who inoculated butter with *Mucor mucedo* whose mycclium penetrated very deeply into the sample. After one years storage at room temperature, noticeable changes were evident in the usual analytical values—

	Original butter-fat.	Blank control.	Moulded sample.
Acid figure (c.c. $\frac{N}{1}$ alkali/100 grs.) .	5·10	107·0	109·5
Reichert-Meissl value . . .	27·17	24·3	24·0
Saponification value . . .	226·00	222·1	217·3
Iodine number	36·20	34·1	35·2
Per cent. volatile acids . .	5·10	4·96	4·90
Molar weight of volatile acids .	93·70	102·1	102·2
Per cent. free volatile acids . .	—	0·40	0·55
Molar weight of free volatile acids	—	154·0	144·0
Ether figure	—	162·2	156·3

The glycerides of the butter-fat were decomposed, yielding glycerol and fatty acids; the glycerol then serves as a nutrient for the moulds to develop further. Most probably the free fatty acids of lower molecular weight are reduced, a hypothesis favoured by the presence of aldehydic compounds in the mouldy sample.

Crampton's summary of the results obtained on the oleo-margarines is very interesting: "The glycerides, exposed to the cool, damp air of the refrigerator, and in contact with the

nitrogenous matter derived from the milk used in the process of manufacture, underwent a gradual decomposition, probably induced and promoted by bacterial growth, with the formation of the lower fatty acids. This progressed to a very considerable degree, giving the high results obtained for the volatile acids and specific gravity obtained from the samples which showed no mould. The germs of the *Coniothecium* fungus having gained access to the fat, or, more probably, having been contained in it originally, and their growth being started by the favourable conditions resulting from the splitting up of the glycerides, attacked the liberated fatty acids and glycerides, and consumed them in proportion to the growth. The growth of the fungus produced further decomposition and a further consumption of fatty acids, with a preference for those of lower molecular weight, until the remaining mixture of fat and free acids gave the diminished values for volatile acids and specific gravity obtained from the moulded samples." [1]

Three samples of oleomargarine stored in sealed cans for three years were examined by Crampton, and the following results obtained—

No.	Initial Reichert-Meissl.	Reichert-Meissl value.	Refractive index.	Specific gravity $\frac{40}{40^\circ}$ C.	Acid value.
1	3·30	4·26	1·4639	0·9102	43·8
2	1·76	5·60	1·4656	0·9159	5·0
3	2·20	5·26	1·4679	0·9144	6·1

The canned margarine exhibited no mould. The specific gravity had evidently increased, since the numbers now approximate to the values obtained for butter-fat.

A study of the fat-splitting bacteria influencing the deterioration of margarines has been made by Söhngen.[2] He showed that milk is a good medium for the development of a large number of fat-splitting bacteria. Their harmful influence depends not only on their lipolytic qualities, but also upon the ill-smelling and bitter-tasting products formed in their action on proteins. The chief micro-organisms investigated were *B. putrificus* (Bienstock), *B. Stutzeri*, *B. vulpinus*, and *B. denitrofluorescens non liquefaciens*. The glycerol and fatty acids, products of lipolytic action, are oxidized by these bacteria.

[1] *Loc. cit.*, p. 718. [2] *Beitr. z. gesamt. Mikrobiol.*, **1**, 199 (1913).

Zoffmann [1] has drawn attention to the deterioration of margarine due to the action of mould fungi and species of saccharomycetes. As a protection from fungi he advises the use of sound parchment paper taken directly from strong brine. For margarine packed in small paper packages, a further precaution is to minimize the air space between the package and the case. A predisposing cause of mould is excess of casein, due to oversoured milk being used. Glucose is to be avoided, as instances are known where the development of carbon dioxide, due to the action of yeasts, has caused the packages to burst.[2]

Tests for Rancidity.—Practically all of the many tests devised to detect rancidity in oils and fats are based on the detection of such products as aldehydes and ketones in the sample examined. Three tests are efficient in detecting rancidity, viz. the Ventilesco-Popesco test, the Kreiss test, and Issoglio's quantitative test. The last two tests are fairly comparable in their detection of the *extent* of the rancidity.

Ventilesco and Popesco [3] argued that rancidity is due to the fixation of oxygen, which could be liberated by the action of peroxidases, and detected by the guaiacum reaction.

Ten grams of the sample are just melted in a test-tube. Now add 5 drops of a 3% solution of hæmoglobin, 10 drops of guaiacum tincture, and 10 c.c. of water. The mixture is well shaken, when, if the oil or fat is rancid, a blue-coloured emulsion will result, the tint-intensity depending on the progress of rancidity. The addition of an equal volume of 95% alcohol to the mixture aids in rendering the blue colour more distinct in samples which are only slightly rancid. The authors point out that the free fatty acidity and rancidity are not necessarily coincident. A rancid fat can be neutralized with alkali and then washed with water and alcohol, when the neutral fat will still answer to the reaction, the blue colour being again of the same intensity as before.

A test devised by Kreiss [4] has been thoroughly investigated by Kerr,[5] who finds the method extremely sensitive and trustworthy. As used in the laboratories of the Meat Inspection Department of the Bureau of Animal Industry (U.S.A.) the test is conducted as follows—

[1] *Chem. Rev. Fett. u. Harz. Ind.*, **11**, 7 (1904).
[2] Cf. Berg., *Zeitsch. Nahr. Genussm.*, **24**, 518 (1913).
[3] *Journ. Pharm. Chim.*, **12**, 318 (1915).
[4] *Verhandlungen der Naturforschenden Gesselschaft in Basel*, **15**, 225 (1903–4).
[5] *Journ. Ind. Eng. Chem.*, **10**, 471–5 (1918).

"Ten c.cs. of the suspected oil or melted fat are placed in a large test-tube (8 × 1) and 10 c.cs. of strong HCl (S.G. 1·19) added. The tube is closed with a rubber stopper and shaken vigorously for approximately thirty seconds. 10 c.cs. of a 0·1% solution of phloroglucin in ether are added, and the tube closed and shaken as before. It is then allowed to stand. If the fat is rancid, a red or pink colour will appear in the acid layer. The depth of this colour is roughly, but not exactly, proportional to the degree of rancidity. To determine the intensity of the reaction, the original fat is diluted with kerosene, and the intensity judged by the degree of dilution at which a reaction ceases to be observed. In judging this point, a recognizable red or pink shade is regarded as a reaction; a faint orange or a yellow tint is not. The intensity of the reaction is reported in terms of the highest dilution at which a reaction is obtained. For example, if a fat is found to react when so diluted that there is one part of fat in twenty parts of the mixture, but not in higher dilution, it is reported as acting in dilution 1–20."

Four classes of fats are recognized by this scheme—

Class I.—Fats giving no reaction. Such fats are sweet and of the highest quality for edible purposes.

Class II.—Fats yielding a reaction when undiluted, but not reacting in dilution 1–10. These fats are not rancid to the taste or smell, but the chemical changes involved in rancidity are definitely in progress.

Class III.—Fats showing a reaction in dilution 1–10, but not in dilution 1–20. This indicates incipient rancidity, evident by taste and smell.

Class IV.—Fats answering to the reaction in a dilution of 1–20 are definitely rancid.

An attempt to measure *quantitatively* the extent of rancidity in oils and fats has been made by Issoglio,[1] who based his method on determining the number of milligrams of oxygen necessary to oxidize the steam distillate from 100 grams of the sample. $KMnO_4$ is used for the oxidation. The method as now practised determines the *total water-soluble oxidizable bodies*, and a quantity known as the "Oxidizability Value" expresses "the number of milligrams of oxygen required to oxidize the organic compounds, separated under constant conditions from the fat." Kerr[2] prefers the following procedure for this test—

[1] *Atti R. Accad. Sci. Torino*, **51**, 582 (1916). [2] *Loc. cit.*, p. 474.

Twenty-five grams of the oil or fat are weighed into a 200 c.cs. Erlenmeyer flask, and 100 c.cs. of distilled water added. The mixture is placed on the steam bath for two hours with frequent shaking. Filter through a wet paper into a 100 c.cs. graduated flask. Cool and make up to the mark with distilled water. 10 c.cs. are pipetted out, diluted with 50 c.cs. of water, and 10 c.cs. of 20% sulphuric acid added. After the addition of 50 c.cs. of $\frac{N}{100}$ potassium permanganate solution, the mixture is boiled for five minutes, using a reflux condenser. Cool, and add 50 c.cs. of $\frac{N}{100}$ oxalic acid, and titrate with $\frac{N}{100}$ potassium permanganate.

If N = number of c.cs. of $KMnO_4$ used in the oxidation,
n = number of c.cs. of $KMnO_4$ used in a blank test,
w = weight of fat used in grams.

then the Oxidizability Value $= \frac{(N-n) \times 80}{w}$.

A value of fifteen or upwards strongly confirms rancidity, and would agree with a Kreiss test answering at dilution 1–20. For normal fats (*e. g.* Classes I and II as above), values ranging from 3–10 are registered.

Use of Preservatives.—Most samples of butter and margarine contain a chemical preserving agent in order to inhibit rancidity and its effects. Common salt alone is an excellent preservative, and is far more efficient than the usually-added boron compounds. Fischer and Gruenert [1] incorporated 3% of salt in margarine, and in three months time the samples were sound, though control samples containing over 1% of boric acid, benzoic acid, and salicylic acid respectively were quite rancid. Similar results have been obtained by the present writer; in one instance a margarine containing 3% of salt was sweet six months later, whilst similar unsalted margarines containing up to 1% of boric acid were moulded throughout. The preserving action of common salt seems to be intimately connected with the accompanying increase in osmotic tension of the aqueous medium.

Boron compounds, particularly boric acid and borax, have long been employed as preservatives for margarines. Their efficiency in this respect is no longer a matter of doubt. Both substances are but weak antiseptics, and for the complete inhibi-

[1] *Zeitsch. Nahr. Genussm.*, **22**, 553 (1911).

tion of bacterial growth practically a saturated solution of boric acid is required. Tanner and Funk [1] have shown very clearly how inefficient boric acid was in germicidal action. Apart from this aspect of the question, there is a physiological objection to the use of boron compounds in foods. Many medical authorities are of opinion that serious disturbance to health may result from continued small doses of boron compounds: as much as 0·5–1·0 gram of boric acid may be consumed daily in butter or margarine. The American investigator, Wiley,[2] strongly condemns the practice.

Formalin has been shown to inhibit very efficiently the development of acidity in butter.[3] But its use in foods is not to be recommended, since it tends to render the protein constituents of foods more indigestible, due to its hardening or "fixing" effect. Sodium carbonate and sodium nitrate have been proposed as preservatives, but Bordas [4] found that rancidity still progresses, although no increase in free fatty acidity occurs. Benzoic acid, benzoates, salicylic acid, and salicylates are sometimes used in margarines as preservatives, but their physiological action is decidedly harmful, irritant effects on the stomach and kidneys resulting.[5]

Patented compounds containing sulphites or bisulphites are sometimes recommended as preservatives in margarine. A mixture containing sodium bisulphate and sodium bisulphite has been proposed, one ounce being sufficient to preserve 1 cwt. of butter or margarine. There is no doubt as to the remarkable preserving powers of sulphites, even in minute quantities, but their use on medical grounds is to be discountenanced. Acute gastric derangement follows the continued ingestion of sulphites, and in feeding experiments on animals, sulphites produce inflammation of the kidneys.

Fluorides act as preservatives, and sodium fluoride has been patented [6] as a margarine and butter preserving agent. Again acute gastric derangement and heart depression may follow the continued use of fluorides, which have not received general favour in commercial practice.[7]

A novel preservative for butter has been patented, viz. gum

[1] *Amer. Journ. Pharm.*, **91**, 206–10 (1919).
[2] U.S. Dept. Agric., Bur. of Chem., *Bull.*, **84**, Part I. (1905).
[3] *Chem. Zeit.* (1895), p. 142.
[4] *Ann. fals.*, **7**, 45 (1914).
[5] Loc. cit., *Bull.*, **84**, Part II. (1906), and *Bull.*, **84**, Part IV. (1908).
[6] Eng. Pat. 3830 (1897).
[7] Cf Vallée, *Journ. Pharm. Chim.*, **21**, 5–8 (1920).

arabic.[1] It is claimed that "the rôle of the gum arabic is to absorb by its dissolubility a great part of the liquid (water or milk) which the treated matter contains, and as gum arabic and its concentrated solutions are not susceptible of fermentation, the fatty matters with which it is mixed are in an unfavourable environment for the development of microbes."

The present trend of opinion is decidedly against the use of any preservatives, other than salt, in butter and margarine, for undoubtedly any reagent possessing an antiseptic action on micro-organisms will be expected to have some action, even if slight, on the enzymes and bacteria in the normal digestive tract of a man.[2]

[1] Eng. Pat. 7620 (1901).
[2] U.S. Dept. Agric., Food Inspection Division, No. **76** (1907).

CHAPTER XII

LARD COMPOUNDS

WITHIN recent years mixtures of beef-fats (stearine and premier jus) and liquid vegetable oils, notably cottonseed oil, have been made so as to resemble lard. The white product, of a granular texture like lard, is used in cooking. The mixture of hard fats and liquid oils is melted and well agitated, and then cooled down as rapidly as possible. Special machinery, somewhat similar to the chilled drums used for margarine, has been

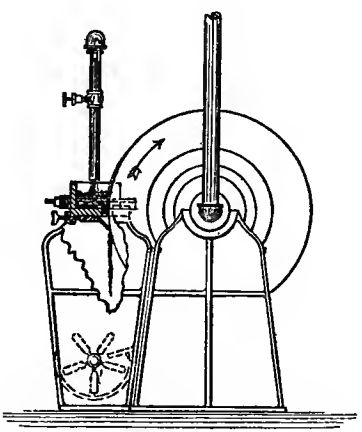

FIG. 20.

devised, particularly in America. Fig. 19 illustrates a modern "chill roll" or "lard cooler."

The upper cylinder is chilled by brine circulation at — 20° to — 12° C., and rotates about 6–10 r.p.m. The melted mixture of fats and oils (at about 50–55° C.) falls into the feeding-trough, 7, and thence on to the chilling roll, there forming a thin translucent layer which rapidly cools and solidifies. A scraper removes the solid fat, which drops into a picker trough, 5. This

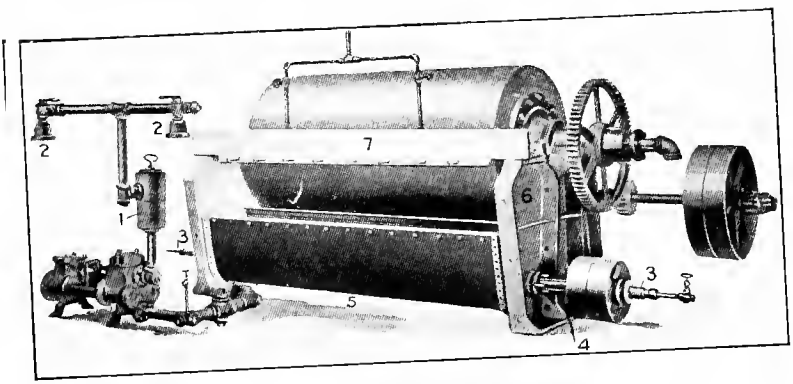

Fig. 19. LARD CHILLING ROLL.

(The Allbright-Nell Co., Chicago.)

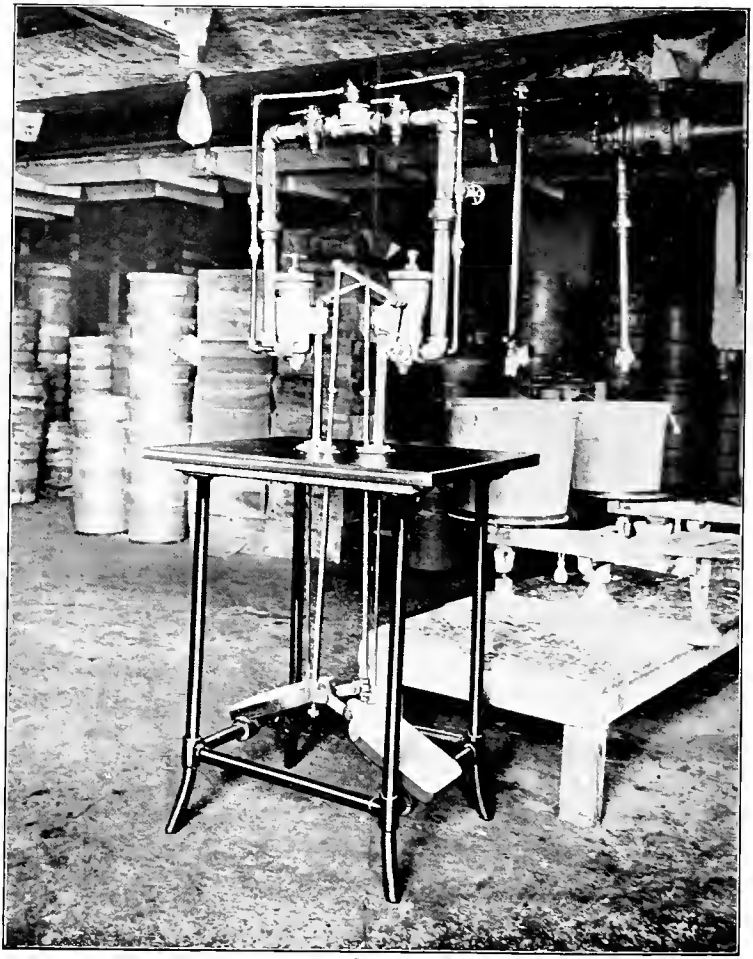

Fig. 21. LARD FILLING MACHINE.

(The Allbright-Nell Co., Chicago.)

trough is provided with a shaft, 4, carrying beating and conveying blades, which serve to beat up the mass and destroy the translucency, imparting a white opaqueness instead. The picker travels at about 180 r.p.m., and the lard is carried towards the pump, which forces it through a strainer, 1, and then through packaging cocks, 2, for delivery into kegs, tins, etc. Fig. 20 is an end view, depicting the chilling roll, feeding-trough, and picker. Fig. 21 illustrates a modern American filling machine for filling the lard compound into one pound cartons.

With the introduction of hydrogenated oils, a new way of making lard compound has been established, viz. the thickening of a single oil, *e. g.* cottonseed oil, by the addition of hydrogen. Even the addition of only 1% of hydrogen to cottonseed oil will lead to a fatty product of lard-like consistency. Instead of partial hardening, the oil may be allowed to absorb hydrogen until a very hard product ensues, and then this may be mixed in suitable proportions with untreated (liquid) cottonseed oil to yield a mixture which cools down to a typical lard compound. Burchenal [1] suggests the incomplete hydrogenation of cottonseed oil to yield a product of iodine value 55–80, melting-point 33–40° C., and titer 35–42° C. A similar idea has been patented by Ellis.[2] Other interesting patents relating to lard compound include those of Winter,[3] Filbert,[4] Ellis,[5] and Mattheiss.[6]

Lard compounds made from hydrogenated products possess a good colour, texture, and flavour, and are particularly stable.

[1] U.S. Pat. 1,135,351 (1915) and 1,135,935 (1915).
[2] *Ibid.*, 1,151,002 (1915).
[3] Eng. Pat. 9526 (1894).
[4] U.S. Pat. 929,925 (1909).
[5] *Ibid.*, 14,607 (1919), reissue of 1,037,881 (1912).
[6] *Ibid.*, 980,809 (1911).

CHAPTER XIII

NUTRITIONAL CHEMISTRY

It has long been recognized that fats are absolutely essential in the human dietary, their deficiency tending to result in such illness as general œdema. With the development of the margarine industry at a time when butter was so scarce, the question of diet assumed an unprecedented importance scientifically as well as economically, and the whole question of margarine and its constituent oils and fats needed serious study and investigation.

Fats supply heat to the organism, and possess the advantages of not being voluminous and of only slowly undergoing digestion. The amount of fat consumed daily by a healthy adult averages 100 grams, and supplies about 28% of the total calories required (total = approximately 3300 calories).

For ready digestion, fats must possess a melting-point not higher than 43° C. Hard fats, *e. g.* stearine (melting-point, 50° C.) are absorbed with difficulty, and about 86–91% passes through the alimentary canal unchanged. Of low-melting fats, such as lard or olive oil, only about 3% escape absorption. If, however, high-melting fats be mixed with other oils and fats in such proportions as to yield a product melting at about 30° C., the mixture becomes readily digestible, so that margarines and butter have practically the same coefficients of digestibility. Jolles,[1] when feeding dogs under similar conditions of dieting, found that from 97·9–98·4% of butter was digested, and from 97·1–97·3% in the case of margarine. He concluded (1894) that oleomargarine could replace natural butter in the diet without detriment. Barterelli,[2] Luhrig,[3] and Hulbgren and Landergren[4] also furnished evidence to show that margarines were assimilated as well as butter.

Similarly Holmes[5] has shown that oleo oil compares very

[1] *Monats. Chem.*, **15**, 147–63 (1894).
[2] *Exper. Stat. Rec.*, xi. 375 (1899).
[3] *Zeitsch. Unters. Genussm.*, **2**, 769 (1900).
[4] Pflüger's Archiv., **83**, 609 (1901).
[5] U.S. Dept. Agric., *Dept. Bull.*, **613** (1919).

favourably with goat's butter, the coefficients of digestibility being 96·8% and 98·4% respectively. Cottonseed oil, a favourite ingredient in most margarines, has been shown to be very readily assimilated, and to be useful in substitution for other oils and fats in cases of chronic dyspepsia.[1]

Fats of the coconut group also compare very favourably with butter-fat.[2] The recently-introduced hydrogenated oils have been shown to be quite satisfactory constituents of margarines, but Fahrion,[3] whilst recognizing their value, believes the evidence is not yet sufficient to show that they are as readily absorbed by the system as ordinary edible fats, such as butter or oleo oil. With hydrogenated whale oil, however, as a specific example, the extensive tests on human beings made by Erlandsen, Fridricia, and Elgström,[4] proved that from 91·6–94·9% was digested (see Chapter III, on "Edible Hydrogenated Oils").

Oils and fats are glycerides, *i. e.* compounds of glycerin with fatty acids, chiefly the normal fatty acids, *i. e.* straight chain compounds containing an even number of carbon atoms. The chief fatty acids thus combined are palmitic acid (C = 16), stearic acid (C = 18), and oleic acid (C = 18). The glycerides occur as mixed glycerides in the oils and fats, and not as simple glycerides, such as triolein, tripalmitin, or tristearin. Thus butter-fat has been shown[5] to contain oleodipalmitin, and butyropalmito-olein. (The chief fatty acids occurring in the individual oils and fats used in margarine will be found in Chapter II, in the discussion under the headings of the various oils and fats.)

The initial seat of fat catabolism, *i. e.* of the breaking up and subsequent chemical changes of the fat, is the liver.[6] From this point onwards the catabolic process is towards complete oxidation to carbon dioxide and water. In the stomach but little change occurs in the fat, such slight hydrolysis as does occur, yielding glycerin and free fatty acids, being due to an enzyme, "gastric lipase."[7] For any appreciable hydrolysis to take place here, the fat must be present in a finely-divided, *i. e.* emulsified, condition. The splitting-up into glycerin and fatty acids

[1] *Chemist and Druggist* (1899), p. 370.
[2] Bourot and Jean, *Comptes rendus*, **123**, 587 (1896). *Vide* also Gardner and Fox, *Biochem. Journ.*, **13**, 368–77 (1919).
[3] *Chem. Umschau.*, **26**, 22, 33 (1919).
[4] *Tidskrift. Kem.*, **15**, 109 (1918).
[5] Amberger, *Zeit. Nahr. Genussm.*, **35**, 313 (1918).
[6] Dakin, *Oxidations and Reductions in the Animal Body* (London, 1912).
[7] Stiles, *Nutritional Physiology* (Philadelphia, 3rd Ed., 1918), p. 89.

is effected mainly by the pancreatic juice (a secretion from the pancreas flowing into the small intestine), together with the bile from the liver. A certain amount of soap is formed, which helps emulsification of the fat. The consideration of the further chemical degradations occurring in the body before the fat is finally converted into carbon dioxide and water is outside the scope of the present volume.

The nutritional chemistry of oils and fats has recently received considerable attention from quite another point of view. Within the last ten years certain new food constituents, too minute in quantity to contribute to the energy supply of the body, have been shown to be absolutely essential to the life of the young growing animal and to the better maintenance of healthy life in the adult animal. These substances, provisionally known as "vitamines," seem to act as catalysts in the normal metabolic processes. The existence of at least three vitamines is now recognized, but so far their chemical constitution is quite unknown, and the isolation of a vitamine in a pure condition has not been accomplished. "There is evidence to suggest that they are formed only in the tissues of plants, whence they pass into the tissues of herbivorous animals, and thus become available for carnivora." [1]

It has now been shown that certain diseases, *e. g.* beri-beri, scurvy, and rickets, are due to some dietary deficiency, viz. a vitamine constituent. Such diseases are termed "deficiency diseases," and a remarkably rapid and permanent cure may frequently be made by providing even a small quantity of the particular vitamine which is lacking. The estimation of the nutritive value of foodstuffs must no longer be based solely on the relative proportions of protein, fat, carbohydrates, and salts which they contain, or on their energy or calorie considerations.

Since the discovery of such indispensable food factors, a voluminous literature has grown, and several synonymous terms have appeared. The word "vitamine" is due to Funk,[2] who distinguished between an antineuritic vitamine, an anti-scorbutic vitamine, etc. Unfortunately the term, though widely accepted to-day, is misleading, since the suffix "-amine," suggests an organic compound of ammonia, and therefore one containing nitrogen. Up to the present, all research tends to suggest that

[1] *Report on Present State of Knowledge concerning Accessory Food Factors (Vitamines)*, (London, 1919), p. 3.
[2] *Journ. State Med.*, **20**, 341 (1912).

the vitamines do *not* contain nitrogen. Other terms proposed have been: "growth substances," "growth determinants," "food hormones," "accessory food factors" (Hopkins), "sitacoids" (Ramsden), and "advitants" (Armstrong).

"Growth substances" and "growth determinants" suggest that vitamines are only essential to growing animals; but they are just as essential to the maintenance of healthy adult life. "Food hormones" is distinctly misleading, since the vitamines are always present in the cells of both animal and plant tissues, whereas hormones are stimulants of certain tissues discharged into the blood-stream from other tissues. Hopkins' term "accessory food factors" is in general use, but it implies that the vitamines are not necessarily indispensable. An accessory food factor, such as spices or mustard, may be omitted from the diet without detriment, whereas vitamines are fundamentally essential. "Sitacoid" means a medicine-like substance associated with food. It is a descriptive term, and does not suggest a definite chemical character.

McCollum and Kennedy[1] have suggested names for the individual vitamines, which are now universally accepted. To the vitamine associated with certain oils and fats they gave the name "fat-soluble A," and to the vitamine never found in fats, but present in milk, yeast, cereals, and certain vegetables, the term "water-soluble B." A third vitamine is now designated "water-soluble C." For the purposes of the present volume, a discussion of "water-soluble B" and "water-soluble C" is not required, since these factors are not associated with fats, and consequently not with margarines. Certainly "water-soluble B" is present in milk, but even then in only a small quantity, and the amount present in either butter or margarine is almost negligible.

"Fat-soluble A," or the anti-rachitic factor, as it is sometimes called, is abundant in butter-fat and cod-liver oil, and also, but to a lesser degree, in cream, mutton-fat, beef-fat (*e. g.* oleo oil), fish oil, liver, kidneys, raw milk, eggs, and the leaves of plants. It is entirely absent in lard, vegetable oils and fats, hydrogenated oils and fats of either animal or vegetable origin, vegetable fats, margarines, and separated milk.

From experiments in animal feeding, notably on rats, certain well-defined results are known to follow a deficiency in the diet of "fat-soluble A," all other food conditions being kept constant

[1] *Journ. Biol. Chem.*, **24**, 491 (1916).

and accurately balanced. At first the young animal maintains normal growth when the "fat-soluble A" is absent, probably because the animal organism has a reserve of this vitamine. Soon, however, the animal no longer increases in weight, and it becomes very susceptible to bacterial infection. In the case of rats, the bacterial infection very frequently leads to an eye disease (xerophthalmia), which rapidly results in severe hæmorrhagic, and, later, purulent discharge, and may lead to blindness. If the diet is changed so as to include an adequate supply of the "fat-soluble A," as, for example, by feeding butter-fat, the eye disease very rapidly disappears and the animal resumes normal growth. Adult animals ostensibly thrive for months on a diet deficient in "fat-soluble A," but sooner or later the general health is impaired, and the resistance to disease greatly diminished. Drummond's recent work has led him to conclude that: "It may be taken as proved that the adult animal organism requires a regular supply of fat-soluble A. This daily requirement is of a much smaller order than that of the young animal, but nevertheless an important factor in the maintenance of health. It appears probable that the resistance to diseases of bacterial origin is seriously impaired by a failure of the animal to obtain a sufficient supply of the fat-soluble A." [1]

The richest sources of "fat-soluble A" are butter-fat and egg-yolk. The vitamine in animal fats is not synthesized by the animal organism, but is derived from the food taken, *e. g.* green leaves and the embryos of certain seeds. McCollum and Simmonds [2] suggest that "fat-soluble A is in chemical union in the plant tissues in a form which is not soluble in ether, and that during digestion or absorption it is set free, and being readily soluble in fats, thereafter accompanies the fat in the animal body."

Of the function of this vitamine in the body we are ignorant. What its fate is after absorption into the system is still unknown, though its appearance in certain depot fats, *e. g.* suet, has led to the idea that it is in part stored in company with reserve fats. It is interesting to note that lack of "fat-soluble A" in the diet does not have any direct influence on the absorption of fat. Drummond [3] showed that rats "are able to absorb large amounts of fatty acids, and presumably synthesize these into fats, in the absence of fat-soluble A in the diet."

[1] *Biochem. Journ.*, **13**, 97 (1919). [2] *Journ. Biol. Chem.*, **33**, 83 (1918).
[3] *Biochem. Journ.*, **13**, 102 (1919).

This vitamine is not soluble in water, but is soluble in alcohol, ligroin, and other fat solvents. It is stable towards dilute acids, but is not soluble therein. Hydrolysis of fats in a non-aqueous medium at room-temperature destroys the vitamine, which has been shown not to correspond to any of the usual components of fats, *e. g.* glycerol, saturated or unsaturated fatty acids, cholesterol, lecithin, phosphatides, or the lipochromes. In the light of our present knowledge, "fat-soluble A" cannot be fractionated into two or more constituents.

The thermostability of "fat-soluble A" is an open question, although recent work by Drummond[1] tends to show that in many cases it may be destroyed by exposure to 100° C. for one hour. Complete but slower destruction is said to follow exposure to temperatures of 50–100° C., and even blood heat (37° C.) for several weeks is said to cause its destruction. Drummond states also that its destruction is apparently not due to oxidation or hydrolysis. During the hydrogenation of oils to yield hard fats used in making margarines and lard-substitutes, the "fat-soluble A" is totally destroyed. Exposure of butter to ultra-violet light destroys the vitamine, and hence the sterilization of whole milk by means of ultraviolet rays is to be advocated with caution, since the nutritive value may be seriously impaired thereby.[2]

It now remains to apply the above statements concerning our present knowledge of "fat-soluble A" to a consideration of the value of margarine in the diet.

Unquestionably, butter is pre-eminent as a source of this dietary-essential. The so-called oleomargarines, containing 50% or so of beef-fats, are excellent substitutes for butter. Their value is, of course, considerably enhanced by admixture with butter itself, from 3% to the legal limit of 10%. Most oleomargarines contain lard, which is remarkable in being apparently the one animal fat devoid of "fat-soluble A." The animal-fat portion, then, of an oleomargarine should not contain lard. Beef-fats alone, particularly oleo oil, are the sole source of this vitamine in margarines. Vegetable oils and fats, as well as hydrogenated oils, are totally deficient in this respect, so that the so-called "vegetable" or "nuts and milk" margarines are not suitable substitutes for butter from this point of view.

[1] *Biochem. Journ.*, **13**, 102 (1919). *Cf.* also *Journ. Biol. Chem.*, **41**, 163–171 (1920).
[2] Zilva, *Biochem. Journ.*, **13**, 164–71 (1919).

In the case of lard-substitutes, of course, the use of vegetable fats and of hydrogenated products does not matter, since the natural lard itself is lacking in the dietary-essential.

The published work [1] on "The Nutritive Value of Margarines and Butter Substitutes, with Reference to their Content of the Fat-soluble Accessory Growth Substance" is due to Halliburton and Drummond, whose conclusions were—

"(1) The fat-soluble accessory growth substance is present in beef-fat and oleo oil, and is present in margarines prepared upon such a basis. Such margarines are nutritively the equivalent of butter.

"(2) Coconut oil, cottonseed oil, arachis oil, and hydrogenated vegetable oils contain little or none of this accessory substance, hence margarines prepared with a basis of these fats have not an equal nutritive value to butter.

"(3) Nut butters prepared from crushed nuts and vegetable fats are similarly not equal to butter.

"(4) Lard substitutes prepared from vegetable oils are equal to lard in their nutritive value, both alike being destitute of the fat-soluble accessory substance."

The accompanying charts are taken from the paper by Halliburton and Drummond, and demonstrate the great superiority possessed by butter and oleomargarines over purely vegetable margarines. Figs. 22 and 23 indicate the growth of rats fed upon oleomargarine and butter respectively, and Fig. 24 the marked decline in growth of rats fed on vegetable margarine; admission of oleomargarine to the diet rapidly restores normal growth.

In conclusion, it is well to recall that butter, owing to the "influence of feed, storage conditions, and temperatures used in the renovation of inferior products, and even the method of use of the product in the home," [2] may not exceed a good oleomargarine in nutritive qualities. The whole question seems to depend upon the adequately-varied and balanced diet of the individual. Children should preferably be fed on butter, but

[1] *Journ. Physiol.*, **51**, 235–51 (1917).
[2] *Journ. Biol. Chem.*, **35**, 517 (1918).

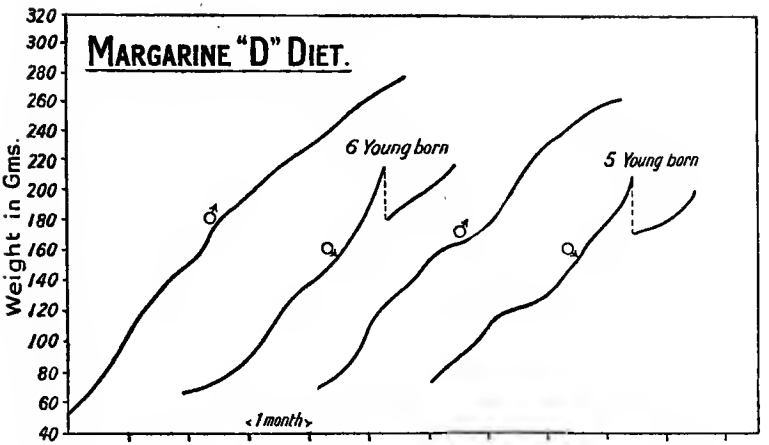

FIG. 22.

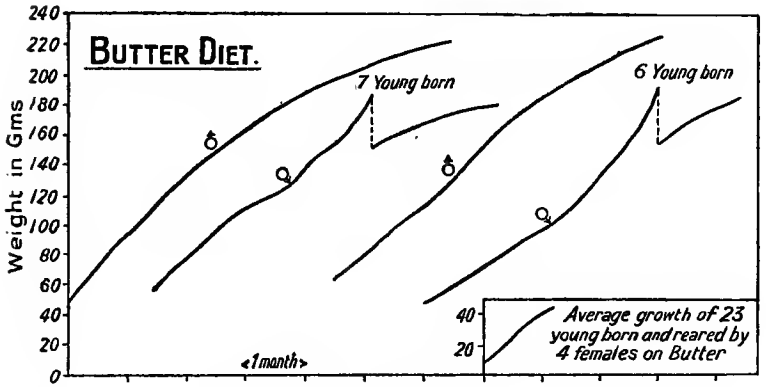

FIG. 23.

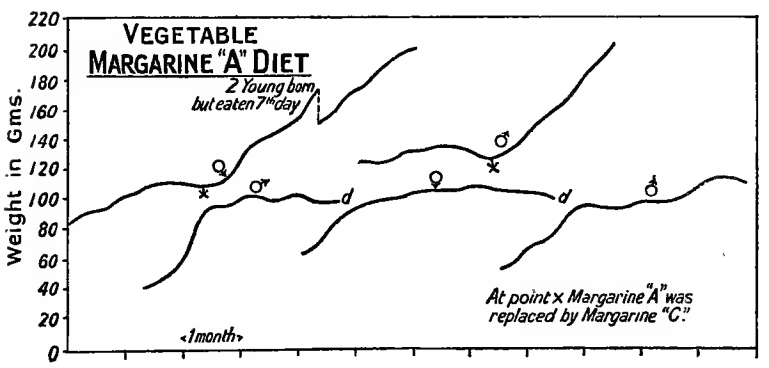

FIG. 24.

adults, with their stronger digestive powers, may with absolute impunity replace butter by either oleo- or vegetable-margarine, provided they consume sufficient amounts of the vegetative green parts of plants, since these furnish an ample supply of all three vitamines.

APPENDIX

"DENATURING" OF MARGARINE

THOUGH butter and margarines may be readily differentiated by a determination of the Reichert-Meissl-Polenské values, legislators in various countries have sought to ear-mark margarines by some simple and rapid test not requiring skilled attention. A popular idea was to add a "latent" colouring matter which could rapidly be made manifest.

Phenolphthalein was proposed by Soxhlet as a convenient latent colour which could be developed by alkali. He recommended 0·3 gram of phenolphthalein per 13·6 lbs. of margarine. Hart [1] showed that no harmful effect on the human system would follow so small a dose as would ordinarily be taken, although larger amounts (*e. g.* a dose of 0·2–0·5 gram) would cause slight diarrhœa. Henriques [2] objected to the use of phenolphthalein on the ground that it would be ineffective owing to the ready solubility of the alkali compounds of this indicator in water.

Turmeric was proposed by Wauters [3] as a suitable latent colour, but this, like phenolphthalein, has not been adopted. Partheil [4] recommended dimethylamido-azo-benzene in the proportion of 1 gram per 1000 kilos of margarine. Such a latent colour would be readily developed as a rose-red hue, with mineral acids or with potassium bisulphate and a little alcohol. Partheil quotes Munk as an authority that this dye is physiologically harmless. Another advantage is its slight solubility in water. The only legal enforcement of dimethyl amido-azobenzene in margarines is in Hungary, where 0·1 gram of this dye must be added to every 100 kilos of margarine.

An excellent ear-marking substance for margarine is potato-starch, proposed by Mainsbrecq,[5] who pointed out that not only

[1] *Chem. Zeit.*, **17**, 1908–9 (1893).
[2] *Chem. Rev. Fett. Harz. Ind.*, **4**, 68–70 (1897).
[3] *Bull. Assoc. Belge des Chim.*, **12**, 58–69 (1898).
[4] *Chem. Zeit.*, **21**, 255–6 (1897).
[5] *Bull. Assoc. Belge des Chim.*, **12**, 185–7 (1898).

would the iodine reaction readily detect its presence, but that also a microscopical examination could be made. According to Mainsbrecq starch will not be washed out of a margarine provided it is quite dry and mixed with the melted fat prior to churning. In Belgium margarines must contain two parts of dry starch per 1000 parts of fat. No other country has adopted this method of indicating margarine.

Most Continental countries now enforce the addition of sesamé oil to margarines. Ten per cent. of this oil is a compulsory ingredient of margarines made in Austria, Sweden, and Denmark. In Belgium it is legal to add fifty parts of sesamé oil during churning. The German law requires that a margarine shall contain sufficient sesamé oil to yield a distinct red colour, when 0·5 c.c. of the clear melted fat in 9·5 c.c. of cottonseed oil is shaken with 10 c.c. of strong hydrochloric acid and a few drops of 2% alcoholic furfural solution.

The use of sesamé oil as a latent colour or ear-marker is recommended because of the ease with which the Baudouin test is made. Owing, however, to the introduction and subsequent extensive employment of artificial colouring agents, the Baudouin test is no longer a delicate and infallible guide to the presence of sesamé oil. Even in 1897, von Raumer [1] showed that such dyes as Ponceau, Orange II, β-naphthol-orange, acid yellow G, Tropäolin OOO No. 2, dimethylaniline orange, and metanil yellow S, all gave, with hydrochloric acid alone, the specific colour yielded by a true Baudouin reaction. No really satisfactory method of detecting sesamé oil in a margarine containing colouring matters yielding a red colour with strong hydrochloric acid has yet been devised.[2]

Although not connected with the denaturing of margarine, certain other legal conditions are prescribed in the different countries. Butter is permitted as an ingredient in margarines if desired, but a limit of 10% is fixed in the British Isles, Belgium and France. In Denmark 15% is permitted, whilst in Germany and Austria only 3·5% of butter is permitted in a margarine.

According to the Butter and Margarine Act of 1907,[3] margarine made in the British Isles must not contain more than 16·0% of water, or more than 0·5% of boron compounds expressed in terms of boric acid. Margarine factories must be registered,

[1] *Zeits. angew. Chem.* (1897), pp. 749–51.
[2] See Fendler, *Chem. Revue* (1905), p. 10, Arnold, *Zeits. Nahr. Genussm.*, **26**, 655 (1913).
[3] Butter and Margarine Act, 7 Ed. VII, c. 21.

and Government inspection maintained. "Margarine" in this connection means "any article of food, whether mixed with butter or not, which resembles butter, and is not milk-blended butter."

Such preservatives as formalin, sodium benzoate, benzoic acid, and salicylic acid are prohibited by the Act. A legal limit for boric acid is also fixed in Italy (0·2%), whilst in Argentina it is prohibited entirely. No preservative of any kind except common salt is allowed in Danish margarine. Amongst other legal points it may be mentioned that artificial colouring agents are prohibited in Italy, Australia, and in the United States of America. According to the Dutch law, no margarine is to be sold which possesses a Reichert-Meissl value exceeding 10·0.[1]

To protect the public from fraud, it is required in the British Isles to attach a label to any package of margarine exposed for sale, on which the word "margarine" is to be printed in 1½ in. letters. If the packages are exposed for sale in a box, it will suffice for the word "margarine" to be printed on the lid, and in that case the packages must be handed to the buyer enclosed in a wrapper bearing only the one word "Margarine," in ½ in. block letters.

[1] *Zeits. Nahr. Genussm.*, **17**, 679 (1909).

BIBLIOGRAPHY

CHAPTER I

INTRODUCTION

ANON. *Oleomargarine.* Nature, **25**, 269 (1882).

——. *Oleomargarine Manufacture.* Eng. & Min. J., **47**, 210–12 (1889).

——. *Modern Substitutes for Butter.* Nature, **95**, 145–7; 372–3 (1915).

——. *Some Problems of the Margarine Industry.* J.S.C.I., **37**, 165–6, R. (1918).

——. *The Modern Technology of Oleomargarine.* Rev. prod. chim., **21**, 40–1 (1918).

——. *The Modern Manufacture of Oleomargarine.* Bol. soc. fomento fabril (Chile), **35**, 529 (1918).

CALDWELL (G. C.). *Oleomargarin.* J. Frank. Inst., **134**, 190–219 (1892).

CLAYTON (W.). *Modern Margarine Technology.* J.S.C.I., **36**, 1205–9 (1917).

——. *Manufacture of Oleomargarine.* Sc. Amer. Suppl., **86**, 182 (1918).

——. *Colloid Problems of Dairy Chemistry.* Brit. Assoc. Colloid Reports, II., 96–117 (1918).

JURGENS (A.). *Butterine or Oleomargarine Butter.* J. Soc. Arts, **33**, 82–7 (1884).

KEEBLER. *Churning Oleomargarine.* Amer. Food J., **13**, 363–7 (1918).

LEE. *Modern Methods of Crystallizing Margarine Emulsion.* Amer. Food J., **13**, 382–4 (1918).

LINDET (L.). *Margarine and Other Edible Fats.* Rev. Scientifique, June 1916, p. 372.

MCGILL (A.). *The Chemistry of Oleomargarine.* Canadian Chem. J., I., 75–7 (1917).

MOLLER (R.). *The Making of Nut Margarine.* Grocery, **16**, 73–7 (1914).

PICKARD. *The Manufacture of Nut Margarine.* Amer. Food J., **13**, 16–19 (1918).

RICHE. *Margarine, a Substitute for Butter and Lard.* Journ. Pharm. **2**, 125–31 (1881).

SCHOORL (N.). *Oleomargarine.* Pharm. Weekblad., **53**, 1316–33 (1916).
SCHRODT (M.). *Artificial Butter from a Hygienic Standpoint.* Bied. Centr., **16**, 270 (1887).
VILLON (A. M.). *Margarine Manufacture in France.* Monit. prod. chim. (1896), p. 4.
WATSON (W. G.). *The Manufacture of Margarine in the United Kingdom.* J. Roy. Soc. Arts, **66**, 212–20 (1918).

BOOKS

BUDDINGER (VON TH.). *Die Margarine Fabrikation.* Separat-Abdruck aus dem Organ für dem Oel und Fetthandel. Verlag. N. Besselich.
GIRARD and BREVANS. *La Margarine et le Beurre Artificiel.* (Paris, 1889.)
LEWKOWITSCH (J.). "Butter Substitutes," in *Chemical Technology and Analysis of Oils, Fats and Waxes* (Macmillan, London, 1915), Vol. III., pp. 25–52.
MITCHELL (C. A.). *Edible Oils and Fats* (Longmans, London, 1918), Chapter IX.

CHAPTER II

OILS AND FATS USED IN MARGARINE MANUFACTURE

ARACHIS OIL

BELLIER (J.). *Detection and Estimation of Arachis Oil.* Ann. Chim. Anal. **4**, 4 (1899).
EVERS (N.). *Detection and Estimation of Arachis Oil.* Analyst, **37**, 487, 537 (1912).
HEIDUSCHKA (A.) and FELSER (S.). *Fatty Acids of Arachis Oil.* Zeits. Nahr. Genussm., **38**, 241–65 (1919).
KERR (R. H.). *Detection of Arachidic Acid.* J. Ind. Eng. Chem., **8**, 904 (1916).
KREIS (H.) and ROTH (E.). *Hardened Oils and the Detection of Arachidic Acid.* Zeits. Nahr. Genussm., **25**, 81 (1913).
MAYER (H.) and BEER (R.). *The Composition of Arachis Oil.* Monatsch. Chem., **34**, 1195 (1913).
SADTLER (S. P.). *Earthnut Oil.* Amer. J. Pharm., **69**, 490 (1897).
SMITH (W. B.). *The Application to Solid Fats of Renard's Test for Peanut Oil.* J. Amer. Chem. Soc., **29**, 1736 (1907).
TORTELLI (M.) and RUGGERI (R.). *The Detection of Cotton, Sesamé, and Arachis Oil in Olive Oil.* Chem. Zeit., **22**, 600 (1898).

Bassia Tallow

Bassia Kernels and Fats. Bull. Imp. Inst., **9**, 228 (1911).

Berg (P.) and Angerhausen (J.). *Unsaponifiable Constituents of Mowrah Fat and the Detection of this Fat in Animal and Vegetable Fats.* Zeits. Nahr. Genussm., **27**, 723 (1914).

Brooks (C. J.). *A Note on Enkabang and Teglam Fats and Katio Oil from Sarawak.* Analyst, **39**, 205 (1909).

Jean (F.). *The Characteristics of Karité Butter.* Ann. Chim. Anal., **11**, 201 (1906).

Sprinkmeyer (H.) and Diedrichs (A.). *Analytical Values of Some Vegetable Fats.* Zeits. Nahr. Genussm., **23**, 581 (1912).

Coconut Oil

Haller (A.) and Youssouffian. *Alcoholysis of Coconut Oil.* Comptes rendus (1906), p. 803.

Haller (A.) and Lassieur (A.). *Occurrence of Odorous Substances in Coconut Oil.* Comptes rendus (1910), p. 1013.

Matthes (H.) and Ackermann (E.). *The Unsaponifiable Constituents of Coconut Oil.* Berichte, **41**, 2100 (1908).

(See also numerous references under "Analysis of Butter and Margarine.")

Cottonseed Oil

Becchi Test. See "Cottonseed Oil" in a paper on *Oils* by G. Negri and G. Fabris. Zeits. anal. Chem. **33**, 560 (1894).

Bömer (A.). *The Detection of Cottonseed Oil in Lard by Means of Phytosterol.* Zeits. Nahr. Genussm., **1**. 544 (1898).

Fischer (K.) and Peyan (H.). *Note on Halphen's Test for Cottonseed Oil.* Zeits. Nahr. Genussm., **9**, 81 (1905).

Fulmer (E.). *Notes on Halphen's Test for Cottonseed Oil.* J. Amer. Chem. Soc., **24**, 1148 (1902).

Garnier (L.). *A Modification of Halphen's Reaction.* J. Pharm. Chim., **29**, 273 (1909).

Gastaldi (E.). *A Modification of Halphen's Test for Cottonseed Oil.* Ann. Lab. Chim. Gabelle, **6**, 601 (1912).

——. *Notes on Halphen's Reaction for Cottonseed Oil.* Ann. Chim. Applic., **2**, 203 (1914).

Halphen (G.). *A Characteristic Reaction of Cottonseed Oil.* J. Pharm. Chim., **6**, 390 (1897).

Holde (D.) and Pelgry (R.). *The Halphen Reaction for Cottonseed Oil.* Chem. Rev. Fett. Harz. Ind., **6**, 67 and 94 (1899).

Marcille (R.). *Detection of Cottonseed Oil in Olive Oil.* Ann. Falsific., **3**, 255 (1910).

Matthés H.) and Heintz (W.). *The Unsaponifiable Substances (in Cottonseed Oil).* Arch. Pharm., **247**, 161 (1909).

PETKOW (N.). *The Detection of Cottonseed Oil.* Zeits. öffentl. Chem., **13**, 21 (1907).
RAIKOW (P. N.). *The Becchi and Halphen Colour Reactions for Cottonseed Oil.* Chem. Zeit., **23**, 1025 (1899).
ROSENTHALER (L.). *Notes on Halphen's Reaction for Cottonseed Oil.* Zeits. Nahr. Genussm., **20**, 453 (1910).
RUPP. *Halphen's Reaction for Cottonseed Oil.* Analyst, **32**, 95 (1907).
UTZ. *A Modification of Halphen's Test for Cottonseed Oil.* Chem. Rev. Fett. Harz. Ind., **20**, 291 (1913).

LARD

ALLBRIGHT (W. B.). *Practical Considerations in Lard Manufacture.* Published by Allbright-Nell Company, Chicago, October 12, 1909.
BELFIELD (W. T.). *The Detection of Beef Fat in Lard.* Analyst, **13**, 70 (1888).
BÖMER (A.). *Mixed Glycerides of Palmitic and Stearic Acid in Lard.* Zeits. Nahr. Genussm., **25**, 321 (1913).
BÖMER (A.), LIMPRICH (R.), KRÖNIG (R.) and KUHLMANN (J.). *The Detection of Beef Fat in Lard.* Zeits. Nahr. Genussm., **26**, 559 (1913).
BÖMER (A.), KRÖNIG (R.), LEY (R.) and MERTEN (H.). *The Detection of Beef Fat in Lard.* Zeits. Nahr. Genussm., **27**, 153 (1914).
FISCHER (K.) and WEWERINKE (J.). *The Detection of Beef Fat in Lard by Polenské and Bömer's Methods.* Zeits. Nahr. Genussm., **27**, 361 (1914).
FULMER (E.). *On the Reaction of Lard from Cottonseed Meal-fed Hogs with Halphen's Reagent.* J. Amer. Chem. Soc., **26**, 837 (1904).
HALFPAAP (G.). *The Relationship between the Iodine Value and the Refraction of Lard.* Zeits. Nahr. Genussm., **15**, 65 (1908).
HEHNER (O.) and MITCHELL (C. A.). *Examination of Lard Crystals Separated from Ether.* Analyst, **21**, 328 (1896).
KREISS (H.) and HAFNER (A.). *Mixed Glycerides in Lard and Beef and Mutton Fat.* Berichte, **36**, 2766 (1903).
——. *The Composition of Lard and Beef Fat Crystals.* Zeits. Nahr. Genussm., **7**, 641 (1904).
LEYS (A.). *The Detection of Foreign Fat in Lard.* J. Pharm. Chim. (1907), p. 289.
MEYER (W.). *Critical Examination of the Iodine Value of Lard.* Zeits. öffentl. Chem., **3**, 134 (1897).
POLENSKÉ (E.). *The Detection of Certain Animal Fats in Admixture with other Animal Fats.* Arb. Kais. Gesundh., **26**, 445 (1907).

RAUMER (VON). *The Examination of American Lard.* Zeits. angew. Chem., **10**, 210; 247 (1897).
SHREWSBURY (H. S.). *A Rapid Sorting Test for the Detection of Paraffin Wax in Lard.* Analyst, **34**, 348 (1909).
SOLTSIEN (P.). *The Halphen Reaction and American Lard.* Zeits. öffentl. Chem., **7**, 140 (1901).
SPRINKMEYER (H.) and DIEDRICHS (A.). *The Detection of Beef and Mutton Fats in Lard by Bömer's Method.* Zeits. Nahr. Genussm., **27**, 571 (1914).
STOCK (W. F. K.). *On the Estimation of Beef Fat in Lard.* Analyst, **19**, 2 (1894).
TOLMAN (L. M.). *The Examination of Lard from Pigs Fed on Cottonseed Meal.* J. Amer. Chem. Soc., **27**, 589 (1905).
WILEY (H.). *Lard and Lard Adulterants.* U.S. Dept. Agric. *Bull.* **13** (1889).
VOIGTLÄNDER. *American Lard.* Zeits. angew. Chem., **11**, 857 (1898).

MAIZE OIL

GILL (A. H.) and TUFTS (C. G.). *Sitosterol: A Possible Test for Maize Oil.* J. Amer. Chem. Soc., **25**, 254 (1903).
VULTE (H. T.) and GIBSON (H. W.). *Corn Oil.* J. Amer. Chem. Soc., **22**, 453 (1900).
WILLIAMS (R.). *Maize Oil.* Analyst, **25**, 146 (1900).

OLEO STEARINE AND OLEO OIL

BÖMER (A.). *Occurrence of Tristearin in Beef and Mutton Tallow.* Zeits. Nahr. Genussm., **14**, 90 (1907).
KREIS (H.) and HAFNER (A.). *The Composition of Lard and Beef Crystals.* Zeits. Nahr. Genussm., **7**, 641 (1904).
FARNSTEINER (K.). *The Separation of Unsaturated Fatty Acids from One Another.* Zeits. Nahr. Genussm., **2**, 25 (1899).

PALM OIL AND PALM KERNEL OIL

BALLAUD. *Rapid Changes in Palm Oil.* Comptes rendus, **167**, 673–4 (1918).
BOLTON (E. R.) and HEWER (D. G.). *Some Brazilian Oil Seeds.* Analyst, **42**, 35 (1917).
BRAY (G. T.) and ELLIOTT (F. L.). *Some New Oil Seeds Derived from American Palms.* Analyst, **41**, 298 (1916).
CRAMPTON (G. A.) and SIMONS (T. D.). *Palm Oil in Butter.* Analyst, **30**, 250 (1905).
——. *The Detection of Palm Oil in Margarine.* J. Amer. Chem. Soc., **27**, 270 (1905).
EATON and SPRING. *African Palm Oil.* Agric. Bull., Fed. Malay States, **6**, 493–512 (1918).

ELLIS (R. H.) and HALL (E. M.). *The Iodine Value (Wijs) of Palm Kernel Oil.* J. Soc. Chem. Ind., **38**, 128 (T.), (1919).
FRANÇOIS. *Oil-producing Palms of French West Africa.* Bull. office colonial, **10**, 223–69 (1917).
GILL (A. H.). *The Crampton-Simons Colour Test for Palm Oil.* J. Ind. Eng. Chem., **9**, 136 (197).
HÉBERT (A.). *The Characteristics of Palm Oil from Various Kinds of Palms.* Bull. Soc. Chim., **9**, 1083 (1911).
HEIDUSCHKA (A.) and BURGER (A.). *Palm Kernel Oil.* Zeits. öffentl. Chem., **20**, 361 (1914).
HEVIN (VON). *The Analysis of Palm Fat.* Chem. Weekblad., **15**, 1394–5 (1918).
LINDET AND DYBROWSKI. *Manufacture of Neutral Palm Oil.* Comptes rendus acad. agric., **5**, 158 (1919).
" L. K." *Palm Oil Bleaching.* Seifensieder Zeit., **40**, 687 (1913).

SESAMÉ OIL

BELLIER (J.). *Two New Colour Reactions for Sesamé Oil.* Chem. Zeit., **23**, 802 (1899).
BREINL (F.). *Some New Colour Reactions for Sesamé Oil.* Chem. Zeit., **23**, 647 (1899).
FLEIG (C.). *Colour Reactions of Sesamé Oil with Aromatic Aldehydes.* Ann. Chim. Anal., **14**, 132 (1909).
KERP (W.). *On the Baudouin Reaction.* Zeits. Nahr. Genussm., **2**, 473 (1899).
KREISS (H.). *Bishop's Reaction for Sesamé Oil.* Chem. Zeit., **23**, 802 (1899).
——. *Some Colour Reactions of Fatty Oils.* Chem. Zeit., **26**, 897 (1902).
——. *Some Colour Reactions of Sesamé Oil. Sesamol a Constituent of Sesamé Oil.* Chem. Zeit., **27**, 108 (1903).
SCHNELL (J.). *Detection of Sesamé Oil in Arachis Oil.* Zeits. Nahr. Genussm., **5**, 961 (1902).
SWAVING (A. J.). *The Detection of Sesamé Oil in Butter from Cows Fed on Sesamé Oil.* Zeits. Nahr. Genussm., **6**, 107 (1903).
VILLAVECCHIA and FABRIS (G.). *Furfural as a Reagent for Sesamé Oil in Other Oils.* Zeits. angew. Chem. (1893), p. 505.

SOYA OIL

BRIGHTMAN (R.). *Note on a Deposit in Refined Soya Bean Oil.* J. Soc. Chem. Ind., **38**, 120, T (1919).
KIESSELBACH. *Soy Beans.* Nebraska Agr. Expt. Stat., *Bull.* **166** (1918), 16 pp.
MORSE. *Soy Bean Industry in U.S.A.* Bur. Plant Ind., Dept. Agr. Year Book, 101–11 (1917).
THURSTON. *Soy Bean Oil.* Midland Druggist (U.S.A.), **52**, 202–3 (1918).

CHAPTER III

EDIBLE HYDROGENATED OILS

ANON. *Hardened Vegetable Oils in the Edible Fat Industry.* Monats. für Margarin Ind., Seifenfabr., **34**, 181 (1914).

AMBERGER. *A Simple Method for the Detection of Tallow and Hydrogenated Fats in Butterfat.* Zeits. Nahr. Genussm., **31**, 297–308 (1916).

ARENTZ (T.). *Reactions in the Hydrogenation of Marine Animal Oils.* Tidsskr. Kem., **16**, 11–13 (1919).

BÖHM (E.). *A Questionable Advance in Margarine Manufacture.* Seifensieder Zeit., **29**, 1087–8 (1913).

BORDAS. *Use of Hydrogenated Oils as Foods.* Ann. Falsif., **12**, 225–35 (1919).

BRAUER (K.). *The Capacity of Hardened Fats for Water, and the Moisture Contents of Margarines Made From Them.* Zeitsch. öffentl. Chem., **22**, 209–16 (1917).

DAVIDSOHN. *Detection of Fish Oils in their Hydrogenated Products.* Seifensieder Zeit., **42**, 657–9, 678–9 (1915).

ELLIS (C). *The Hydrogenation of Oils.* J.S.C.I., **31**, 1165 (1912).

——. *The Hydrogenation of Oils.* (Constable, London, 1914).

ERLANDSEN (A.), FRIDRICIA (L. S.) and ELGSTRØM (A.). *Hardened Whale Oil as Human Food.* Tidskrift Kem., **15**, 109–33 (1918).

FAHRION (W.). *Digestibility of Hardened Fats.* Chem. Umschau., **26**, 22, 33 (1919).

GRIMME (C.). *Hardened Marine Animal Oils.* Chem. Rev. Fett. Harz. Ind., **20**, 129 (1913).

——. *Colour Reactions of Hardened Whale Oils. Loc. cit.*, p. 155.

KERR (R. H.). *The Detection of Nickel in Fats.* J. Ind. Eng. Chem., **6**, 207 (1914).

KLIMONT (J.) and MAYER (K.). *Use of Hardened Whale Oil in Oleomargarine Manufacture.* Zeitsch. angew. Chem., **27**, 645–8 (1914).

KNAPP (A. W.). *Note on Hardened Oils.* Analyst, **38**, 102 (1913).

KREISS (H.) and ROTH (E.). *Hardened Oils and the Recognition of Arachidic Acid.* Zeits. Nahr. Genussm., **25**, 81 (1913).

LEHMANN (K. B.). *Are Hardened Fats Suitable for Human Food?* Chem. Zeit., **38**, 798–9 (1914).

MAILHE (A.). *The Hydrogenation of Oils.* J. usines gaz., **43**, 1, 17, 33 (1919).

MOORE (H. K.), RICHTER (G. A.) and ARSDEL (W. B. VAN). *The Incomplete Hydrogenation of Cottonseed Oil.* J. Ind. Eng. Chem., **9**, 451–62 (1917).

NORD (F. F.). *Catalytic Hydrogenation of Fats with Palladium.* Zeits. angew. Chem., **32**, 305–9 (1919).

OFFERDAHL (H. T.). *The Production and Utilization of Whale Oil.* Ber. deut. Pharm. Ges., **23**, 558 (1913).
PEKELHARING and SCHUT. *A Study of the Food Values of Hardened Oils.* Pharm. Weekblad., **53**, 769–85 (1916).
REISS (G.). *Nickel and Arsenic in Hardened Fats.* Arb. Reichsgesundheitsamte, **51**, 521–31 (1919).
SANDELIN (A. E.). *Hydrogenated Fats.* Technikern (1913), p. 350.
THOMAS (R.). *The Mechanism of the Addition of Hydrogen to Unsaturated Glycerides in the Presence of Finely Divided Nickel.* J.S.C.I., **39**, 10–18, T (1920).
THOMAS and MULLER. *The Application of Hardened Fats in the Food Industry.* Arch. Hyg., **84**, 56–77 (1915).
TORTELLI (M.) and JAFFE (E.). *Specific Colour Reactions of Marine Animal Oils and their Hydrogenated Products.* Annali. Chim. Applic., **11**, 80 (1914).
UBELOHDE (L.) and SVANOE (T.). *The Technical Hydrogenation of Fats with Nickel Catalyst.* Zeits. angew. Chem., **32**, 257–62, 269–72, 276–80 (1919).
VIÉ (G.). *Hydrogenation of Oils.* L'indus. chim., **6**, 364–5 (1919).
VOLLERSTEN (J. J.) and OSBORN (A. G.). *A Study of the Titers and Iodine Numbers of Mixtures of Vegetable Stearin (Hydrogenated) and Certain Vegetable Oils.* Cotton Oil Press, **2** (No. xi.), 39–40 (1919).

CHAPTER IV

THE EXAMINATION OF MILK FOR USE IN MARGARINE

AMBERGER (A.). *The Judging of Milk.* Zeits. Nahr. Genussm., **30**, 16–23 (1915).
BARTHEL (C.). *The Reductase Test Compared with other Tests for the Determination of the Hygienic Properties of Milk.* Mon. Sci., **8**, 176–81 (1918).
BARTHEL (C.) and JENSEN (O.). *International Methods of Judging Milk.* Milchw. Zentr., **41**, 417–29 (1912).
BARTIN-SANS (H.) and GANJOUX (E.). *Reductases of Cow's Milk; their Importance for the Hygienic Value of the Milk.* Rev. Hyg., **36**, 258–71 (1914).
BOLTON (E. R.) and REVIS (C.). *Fatty Foods.* (London, Churchill), Chapter IX, "Milk."
BREED. *Bacteriological Methods for Market Milk Analysis.* J. Dairy Sci., I., 380–2 (1918).
BREED and BREW. *The Control of Market Milk by Direct Microscopic Examination.* N.Y. Agric. Expt. Statn. *Bull.* **443**, 717–45 (1917).

BREW and DOTTERER. *The Number of Bacteria in Milk.* N.Y. Agric. Expt. Statn. *Bull.* **439**, 477–522 (1917).

DONS (R.). *The Value of the Reductase or the Fermentation Reductase Test.* Centr. Bakt. Parasitenk., I., Abt. **40**, 132–53 (1914).

FAITELOWITZ (A.). *Catalase in Milk and the Use of its Determination in the Control of Milk Supplies.* Milchw. Zentr., 299–427 (1910).

FRED (E. B.). *A Study of the Quantitative Reduction of Methylene Blue by Bacteria found in Milk, and the Use of this Stain in Determining the Keeping Quality of Milk.* Centr. Bakt. Parasitenk., II., Abt. **35**, 391–428 (1913).

HAMMER (B. W.) and HAUSER (A. J.). *Studies in the Clarification of Milk.* Iowa Agr. Expt. Statn. *Res. Bull.*, **47**, 79–97 (1918).

HARDING, BREED, STOCKING, and HASTINGS. *What is Meant by Quality in Milk?* J. Dairy Sci., I., 199–217 (1917).

HUNTER (O. W.). *Colon-aerogenes Group of Milk.* J. Dairy Sci., II., 108 (1919).

JONA (T.). *The Oxidation-index of Milk.* Chem. Centralb., **2**, 1328 (1910).

LANE-CLAYPON (J.). *Milk and its Hygienic Relations* (Longmans, London, 1916), Chapter XVII, also Appendix G, p. 325.

PARASCHCHUK (S.). *Biological Methods of Determining Milk Quality.* Milch. Zentralb., **42**, 69 (1913).

PERRIRAZ (J.). *The Reducing Enzymes of Milk.* Arch. sci. phys. nat., **46**, 101–2 (1919).

PRUCHA, WEETER, and CHAMBERS. *The Germ Content of Milk as Influenced by the Utensils.* Univ. Ill. Agr. Expt. Statn. *Bull.* **204**, 217–57 (1918).

RACE (J.). *The Examination of Milk for Public Health Purposes* (Wiley, New York, 1918).

SHERMAN and REYNOLDS. *Bacteriological Methods for Determining the Quality of Milk.* Penn. State Coll. Expt. Statn., Ann. Rept. (1914–15), p. 294.

STAPENSÉA (H.). *What is the Best Test for Judging the Quality of Milk?* Tidjschr. Veeartsenijk, **39**, 231–60 (1913).

THILO (H.) and GUILLAUME (A.). *The Rating of the Hygienic Value of Milk as Delivered for Consumption.* Bull. sci. pharmacolog., **26**, 151–8, 268–74 (1919).

VERDA and KIRCHENSTEINS. *Hygienic Milk Control.* Schweiz. Apoth. Zeit., **55**, 665, 677, 689, 705 (1917).

CHAPTER VI

TREATMENT OF THE MILK

PASTEURIZATION AND STERILIZATION

AYERS (S. H.) and JOHNSON (W. T.). *A Study of the Bacteria which Survive Pasteurization.* U.S. Dept. Agric. *Bull.* **161**, (1913).

——— ———. *The Destruction of the Bacteria in Milk by Ultraviolet Rays.* J. Wash. Acad., **3**, 160 (1914).

——— ———. *Ability of Streptococci to Survive Pasteurization.* J. Agric. Res. (1914), p. 321.

AYERS (S. H.). *Present Status of Milk Pasteurization.* U.S. Dept. Agric., Bur. An. Ind., *Bull.* **361** (1916).

BEATTIE (J. M.). *The Utilization of Electricity in the Continuous Sterilization of Milk.* Jour. Path. Bact., **18**, 120 (1913).

———. *The Electrical Treatment of Milk.* Rept. of City Bacteriologist, Liverpool, 1914.

BOUMA (A.) and VAN DAM (W.). *The Effect of the Degree of Acidity of Milk upon the Velocity of Destruction of Peroxidase by Heat.* Biochem. Zeit., **92**, 385–96 (1918).

CAZENEUVE (P.). *Sterilization of Milk, and Lactic Fermentation.* Bull. Soc. Chim., **13**, 502 (1895).

CHICK (H.). *Sterilization of Milk by Hydrogen Peroxide.* Centr. Bakt. Parasitenk., **7** (2), 705–17.

FLÜGGE. *The Problem and Production of Sterilized Milk.* Zeitsch f. Hygiene, **17**, 272–342 (1894).

FREUDENREICH (V.). *The Pasteurization of Milk for Infant Feeding.* Rev. gén. du Lait, 1904–5 (**4**), p. 433.

FREUND (W.). *Action of Ozone on Milk and Dairy Produce.* Chem. Zeit., **35**, 905–6 (1911).

FROST (W. D.). *Microscope Test for Pasteurized Milk.* J. Amer. Med. Assoc., **64**, 821–2 (1915).

FROST (W. D.) and MOORE (G. D.). *The Cellular Test for Pasteurized Milk under Practical Conditions.* J. Dairy Sci., **2**, 189–96 (1919).

GRULEE. *Oxygenated Milk.* N.Y. Med. Jour., **104**, 1092–4 (1916).

HELMHOLTZ. *Pasteurization, Sterilization, and Boiling of Milk.* Ann. Meeting, Amer. Assoc. Prevention of Infant Mortality, 1913 (**3**), p. 206.

JORDAN (E. O.). *The Case for Pasteurization.* J. Amer. Med. Assoc., **59**, 1450–7.

KILBOURNE (C. H.). *The Practical Pasteurization of Milk from the Practical Standpoint* (Wiley, New York, 1916).

KUHL. *Pasteurization of Milk.* Pharm. Zentralhalle, **56**, 595 (1915).

LARSON, HATZELL, and DIEHL. *Effect of High Pressures on Bacteria.* Jour. Infec. Diseases, **22**, 271–9 (1918).

LEWIS (F. C.). *A New Method for the Destruction of Bacteria in Large Volumes of Milk by Means of Electricity.* Jour. Board Agric., **22**, No. 12, March 1916.

LOHNIS (F.). *The Biorisator Process and Enzyma Milk.* Milchw. Zentr., **43**, 59–61 (1914).

MORSE (J. J.). *Sterilization, Boiling, and Pasteurization of Milk.* J. Amer. Med. Assoc., **60**, 875–8.

ROBERTSON and MAIR. *Bacteriology of So-called Sterilized Milk.* Brit. Med. Jour. 1904 (**1**), p. 1122.

ROGERS (L. A.), BERG (W. N.) and DAVIS (B. J.). *The Temperature of Pasteurization for Buttermaking.* U.S. Dept. Agric., Bur. An. Ind., *Circ.* **189**, 307–26 (1912).

ROSENAU. *The Thermal Deathpoints of Pathogenic Microorganisms in Milk.* Dept. of Hygiene, Washington (1908), *Bulls.* **41** and **42**.

——. *Pasteurization. Loc. cit.* (1912), *Bull.* **56**.

SMITH (R. S.). *Observations on the Pasteurization of Milk in City Milk Plants.* J. Dairy Sci., **2**, 487–503 (1919).

ST. JOHN and PENNINGTON. *The Relative Rate of Growth of Milk Bacteria in Raw and Pasteurizcd Clean Milk.* Jour. Infect. Diseases, **7**, 647 (1907).

TIEMANN. *Outlines of Various Methods of Pasteurizing Milk.* Molkerei Zeit., **20**, 53 and 69 (1916).

THORNTON (W. M.). *The Electrical Conductivity of Bacteria, and the Rate of Sterilization of Bacteria by Electric Currents.* Proc. Roy. Soc. [B], **85**, 331–44 (1913).

VARIOT (G.). *Nutritive Value of Cow's Milk Sterilized at* 108° *C.* Comptes rendus, **139**, 1002–3 (1904).

WEBER. *Bacteriology of the So-called Commercial Sterilized Milk.* Arbt. a. d. Kaiserl. Gesundheitsamte, **17**, 108–55 (1900).

STERILIZATION BY ULTRAVIOLET RAYS

AGULHON (H.). *Action of Ultraviolet Light on Enzymes.* Comptes rendus, **152**, 398–401 (1911).

AYERS (S. H.) and JOHNSON (W. T.). *The Destruction of Bacteria in Milk by Ultraviolet Rays.* J. Wash. Acad. **3**, 160.

BOVIE. *The Schumann Rays as an Agent for the Sterilization of Liquids.* Botan. Gaz., **60**, 144–8 (1915).

BROWNING and RUSS. *Germicidal Action of the Ultraviolet Radiation.* Proc Roy. Soc. [B], **90**, 33–8 (1917).

BURGE. *Mode of Action of Ultraviolet Radiation in Producing Sterilization.* J. Frank. Inst., **182**, 264 (1916).

CERNOVODEANU (P.) and HENRI (V.). *Action of Ultraviolet Rays on Microbes.* Comptes rendus, **150**, 52–4 (1910).

ERNST (W.). *Milk Hygiene* (Chicago, 1914), pp. 200–202.

MARCAS (L.) and HUYGE (C.). Discussion of *Sterilization of Milk by Ultraviolet Rays* in :—Ann. Rept. Dairy Div. Agric. Expt. Statn. at Gembloux (Brussels, 1912), p. 70.
RECKLINGHAUSEN (VON M.). *Sterilization of Liquids with Ultraviolet Rays.* Die Umschau (1911), pp. 801–4.
RÖMER (P. H.) and SAMES (T.). *Sterilization of Milk by Ultraviolet Rays.* Chem. Centralb., 2, 1397 (1910).
ZILVA (S. S.). *Action of Ultraviolet Rays on Vitamines.* Biochem. J., **13**, 164–71 (1919).

INOCULATION AND SOURING OF MILK

Ferment Powders

BOUVET (M.). *Rapid Analysis of Lactic Ferment Powders.* Bull. sci. pharmacol., **20**, 483–4 (1914).
CARRION and SOREL. *Possibility of Obtaining the Dried Living Lactic Ferment.* Bull. gén. thérap., **163**, 494 (1913).
HASTINGS (G. E.). "Commercial Cultures," in *Microbiology*, p. 413 (Churchill, London, 1917).
RUSSELL (A. L.) and HASTINGS (G. E.). "Starters," in *Experimental Dairy Bacteriology* (Guin, London, 1909), pp. 97–100.
STOCKING (W. A.). *Manual of Milk Products* (Macmillan, New York, 1917), pp. 239–48, on "Starters."

Lactic Acid Bacteria

BEIJERINCK (W. M.). *Industrial Lactic Acid Bacteria.* Zeits. Spiritusind (1902), **25**, 50, 530, 541, 550.
GORINI (C.). *Distinction of Different Classes of Lactic Acid Bacteria.* Milch. Zentralb. **42**, 417–24 (1914).
HENNEBERG (W.) *Lactic Acid Bacteria.* Woch f. Brau., **18**, 381–4 (1901).
LOHNIS. *Classification and Nomenclature of Lactic Acid Bacteria.* Milch. Zentralb., **45**, 49 (1916).
PARASCHTSCHUK (S.). *Lactic Acid Bacteria in the Dairy Industry.* Milch. Zentralb., **45**, 51–2 (1916).

Lactic Fermentation

BUEL (H.). *Preserving Milk with Lactic Acid Bacteria.* U.S. Pat., 1,327,308 (1920).
DAM (W. VAN). *Influence of Lactic Acid on Lactic Acid Formation.* Biochem. Zeits., **87**, 107–22 (1918).
FINKELSTEIN. *The Study of the Factors Concerned in the Preparation of Milk Fermented with Bacillus Bulgaricus and Bacterium acidi lactici.* J. Dairy Sci., I., 250–8 (1917).
GORINI (C.). *The Proteolytic Activity of Lactic Ferments.* Atti. acad. Lincei, **26** (II.), 195–9, 223–7 (1917).

GRIMM (M.). *The Chief Phases of Lactic Acid Fermentation and their Practical Importance.* Zentr. Bakt. Parasitenk. (1911), **2**, Abt. **32**, 65–70.

HASTINGS (E. G.) and HART (E. B.). *The Presence of an Acid-Producing Enzyme in Bacterium lactis acidi.* Proc. Ann. Soc. Biol. Chem. (1912), p. 38.

HEINEMANN (A.). *Kinds of Lactic Acid Produced by Lactic Acid Bacteria.* J. Biol. Chem., **2**, 603–12 (1907).

LESAGE and DONGIER. *Study of the Lactic Fermentation by Observation of the Electric Resistance.* Comptes rendus, **134** [10], 612–14.

MAKRIPOV (I. A.). *The Effect of the Neutralization of the Nutrient Media with Chalk upon the Activity of Lactic Acid Bacteria.* Zentr. Bakt. Parasitenk., II., Abt. **37**, 609–22 (1913).

NORTHRUP (Z.). *Influence of Certain Acid-Destroying Yeasts upon Lactic Bacteria.* Mich. Agr. Expt. Statn., *Tech. Bull.* **15**, 3–35 (1913).

SCHIERBECK (N. P.). *Variation of the Fermenting Power of Lactic Acid Bacteria.* Chem. Zeit. Rep., Copenhagen (1900), **24** [19], 171.

SLYKE (L. L. v.) and BOSWORTH. *Chemical Changes in the Souring of Milk.* N.Y. Agric. Statn., *Tech. Bull.* **48**, 3–12 (1916).

—— and BAKER (J. C.). *Free Lactic Acid in Sour Milk.* J. Biol. Chem., **35**, 147–78 (1918).

SVANBERG (O.). *Acidity Relations of Lactic Acid Bacteria.* Med. K. Vetensk. Nobelinst., **5**, II., 1–10 (1919).

WEIGMANN (H.). *Relation of Lactic Acid Bacteria to the Heating of Milk to 60–63° C.* Zentr. Bakt. Parasitenk., II., Abt. **45**, 63–107 (1916).

ARTIFICIAL MILK

CHAPIN. *Preliminary Report on the Use of Vegetable Milk.* Arch. Pediatrics., **35**, 365–6 (1918).

CLAYTON (W.). *Artificial Milk.* Brit. Assoc. Comm. Colloid Chem. Rept. **2**, 106 (1919).

GERLEI (L.). *Artificial Milk.* Milch. Zentr., **42**, 49–52 (1913).

GERSTENBERGER (H. J.) and RUH (H. O.). *Studies in the Adaptation of an Artificial Food to Human Milk.* Ann. J. Dis. Children, **17**, 1–37 (1919).

Relevant Patents—

BECKMANN and DYCK. U.S. Pat. 1,216,052 (1917).

VAN DER BERGH. Eng. Pat. 3359 (1882).

BURDICK and NIELSEN. U.S. Pat. 1,273,144 (and 5) [1918].

BURRELL and COOLEY. Eng. Pat. 3348 (1882).

ERSLEV. Eng. Pat. 121,133 (1918). U.S. Pat. 1,297,668 (1919).

GOSSEL. Eng. Pat. 27,860 (1912). Fr. Pat. 451,447 (1912).

LI. Eng. Pat. 30,275 (1910).

Relevant Patents—

LIEBRICH. Eng. Pat. 12,355 (1898).
KAUFMANN. Eng. Pat. 7296 (1913).
MELHUISH. Eng. Pats. 24,572 (1914), 9626 (1915), 118,535 (1917). U.S. Pat. 1,243,855 (1917).
MONAHAN and POPE. U.S. Pat. 1,104,376 (1914).
MOSES. U.S. Pat. 1,281,411 (1918).
PIPERNO. U.S. Pat. 1,267,449 (1918).
VON RIEGLER. Fr. Pat. 461,131 (1913).
SCHLINCK. U.S. Pat. 1,220,010 (1917).
WILSON. Eng. Pat. 240,050 (1911).

CHAPTER VIII

THE THEORY OF EMULSIFICATION

BANCELIN. *Viscosity of Emulsions.* Comptes rendus, **152**, 1382–3 (1911).

BANCROFT (W. D.). *The Theory of Emulsification.* Jour. Phys. Chem., **16**, 177, 345, 475, 739 (1912); **17**, 501 (1913).

BAUER (H.). *Investigation of the Surface Tension Relations in Milk, and Nature of the Membranes Surrounding the Fat Globules.* Biochem. Zeit., **32**, 362–79 (1911).

BELCHIC and NEAL. *Surface Tension of Oil-Water Emulsions.* Mining Eng. World, **45**, 487–9 (1916).

BLOOR (W. R.). *A Method for Determining Fat in Milk* (nephelometer). J. Amer. Chem. Soc., **36**, 1300–70 (1914).

BRIGGS (R. T.) and SCHMIDT (H. F.). *Experiments on Emulsions.* Jour. Phys. Chem., **19**, 478–99 (1915), *vide also*, loc. cit., **24**, 120; 147 (1920).

CLAYTON (W.). *Modern Conception of Emulsions.* J.S.C.I. **38**, 113–18, T (1919).

CLOWES (G. A. H.). *The Action of Electrolytes in the Formation and Inversion of Oil-Water Systems, with Certain Biological Applications.* Koll. Zeitsch., **15**, 123–26 (1914).

——. *Emulsions and Living Cells: Action of Antagonistic Electrolytes.* Jour. Phys. Chem., **20**, 407–51 (1916).

DONNAN (F. G.) and POTTS (H. E.). *On the Emulsification of Hydrocarbon Oils by Aqueous Solutions of Fatty Acid Salts.* Koll. Zeitsch., **9**, 208 (1911).

ELLIS (R.). *The Properties of Oil-Water Emulsions.*
(1) "The Electric Charge." Zeits. phys. Chem., **78**, 321–52 (1912).
(2) "Stability and Size of Particles." Zeits. phys. Chem., **80**, 597–616 (1912).
(3) "Coagulation by Colloidal Solutions." Zeits. phys. Chem., **89**, 145–50 (1914).

ELLIS (R.). *A Neutral Oil Emulsion as a Model of a Suspension Colloid.* Trans. Farad. Soc., **9**, 14–25 (1913).

FANTO and STRITAR. *The Clearing of Emulsions.* J. prakt. Chem., **81**, 564–8 (1910).

FISCHER (M. H.) and HOOKER (M. D.). *On the Formation and Decay of Emulsions.* Koll. Zeitsch., **18**, 129 (1916).

—— ——. *Fats and Fatty Degeneration.* (Wiley, New York, 1917).

GOODWIN and ELLIS. *Electrical Separation of Oil from Condenser Water.* Chem. Zeit., **81**, 724 (1910).

GROSCHUFF (E.). *The Stability of Emulsions of Water in Hydrocarbon Oils.* Koll. Zeitsch., **9**, 257–9 (1911).

HATSCHEK (E.). *The Direct Separation of Emulsions by Filtration and Ultrafiltration.* J.S.C.I., **29**, 3 (1910).

——. *The Filtration of Emulsions, and the Change in Shape of Emulsified Particles under Pressure.* Koll. Zeitsch., **7**, 81–86 (1910).

——. *Emulsions.* Brit. Assoc. Comm. on Colloids Report, **2**, 16–21 (1918).

JAEGER and KAHN. *The Surface Energy of Homologous Triglycerides of the Fatty Acids.* Proc. Acad. Sci., Amsterdam, **18**, 285–96 (1915).

LEHNER (V.) and BUELL (R.). *Studies in Soap Solutions, I.* J. Ind. Eng. Chem., **8**, 701–3 (1916).

—— and BISHOP (G. M.). *Studies in Soap Solutions, II.* Jour. Phys. Chem., **22**, 68–72 (1918).

LEWIS (W. C. Mc. C.). *The Size and Electric Charge of Oil-particles in Oil-in-Water Emulsions.* Koll. Zeitsch., **4**, 211–12 (1909).

——. *The Surface Tension of Colloid and Emulsoid Particles and its Dependence on the Limit Size of the Latter.* Koll. Zeitsch., **5**, 91–93 (1909).

MOORE (W. C.). *Emulsification of Water and of Ammonium Chloride Solutions by Means of Lamp-black.* J. Amer. Chem. Soc., **41**, 940–6 (1919).

MOORE (B.) and KRUMBHOLZ. *On the Relative Powers of Various Forms of Proteids in Conserving Emulsions.* Brit. J. Physiol., **22**, 54 (1908).

NEWMAN (F. R.). *Experiments on Emulsions.* J. Phys. Chem., **18**, 34–55 (1914).

OSTWALD (W.). *Contributions to the Knowledge of Emulsions.* Koll. Zeitsch., **6**, 103 (1910).

PICKERING (S. U.). *On Emulsions.* (*a*) Koll. Zeitsch, **7**, 11–16 (1910); (*b*) J. C. Soc., **91**, 2002–2021 (1907).

POWIS (F.). *Influence of Electrolytes on the Potential Difference at the Oil-Water Boundary of an Oil Emulsion, and at the Water-Glass Boundary.* Zeitsch. phys. Chem., **89**, 91–110 (1914).

——. *Influence of Time on the P.D. at the Surface of Oil Particles Suspended in Water. Loc. cit.*, **89**, 179–85 (1914).

POWIS (F.). *Relationship between the Stability of an Oil Emulsion and the P.D. at the Oil-Water Interface, and the Coagulation of Colloidal Suspensions.* *Loc. cit.*, **89**, 186–212 (1914).

RAMSDEN (W.). *Separation of Solids in the Surface Layers of Solutions and Suspensions.* Zeitsch. phys. Chem., **47**, 336–46 (1904).

——. *Surface Films.* Trans. Liv. Biol. Soc., **33**, 3–24 (1919).

ROBERTSON (T. B.). *Notes on Some Factors which Determine the Constitution of Oil in Water Emulsions.* Koll. Zeitsch., **7**, 7–10 (1910).

ROON (L.) and OESPER (R. E.). *Theory of Emulsification Based on Pharmaceutical Practice.* J. Ind. Eng. Chem., **9**, 156–61 (1917).

SCHLAEPFER (A. V. M.). *Water-in-Oil Emulsions.* J. C. Soc., **113**, 522–6 (1918).

SHEPPARD (S. E.). *Emulsification by Adsorption at an Oil-Water Interface.* J. Phys. Chem., **23**, 634–9 (1919).

SHORTER and ELLINGWORTH. *The Emulsifying Action of Soap.* Proc. Roy. Soc. [A], **92**, 231–47 (1916).

SISLEY. *The Solvent Properties of Emulsions.* Bull. Soc. Chim., **20**, 155–7 (1917).

WHITNEY (W.) and STRAW (A.). *Suspensions in Dilute Alkaline Solutions.* J. Amer. Chem. Soc., **29**, 325 (1907).

WIEGNER (G.). *Emulsion Colloids (Emulsoids) and Observations on the Method of Counting Ultramicroscopic Particles.* Koll. Chem. Beihefte, **2**, 213–42 (1911).

CHAPTER IX

BUTTER AND RENOVATED BUTTER

COMPOSITION OF BUTTERFAT

AMBERGER (C.). *The Glycerides of Butterfat.* Zeits. Nahr. Genussm., **35**, 313–81 (1918).

BROWNE (C. A.). *A Contribution to the Chemistry of Butterfat.* J. Amer. Chem. Soc., **21**, 612–33 (1899).

——. *Chemical Composition of Butterfat.* *Idem.*, **21**, 807–27 (1899).

CALDWELL (K. S.) and HURTLEY (W. H.). *The Distillation of Butterfat, Coconut Oil, and their Fatty Acids.* Proc. Chem. Soc., **26**, 73 (1909).

ENGELEN (A. v.) and WAUTERS (P.). *Contribution to the Chemistry of Butterfat.* Bull. Assoc. Belge des Chim., **13**, 282–90 (1899).

HENRIQUES (R.). *On the Volatile and Insoluble Fatty Acids in Butter.* Chem. Rev. Fett. Harz. Ind., **5**, 169–72 (1898).
HOLLAND (E. B.) and BUCKLEY (J. P.). *Determination of the Fatty Acids in Butterfat.* J. Agric. Res., **12**, 719–32 (1918).
—— REED (J. C.) and BUCKLEY (J. P.). *Estimation of Stearic Acid in Butterfat.* J. Agric. Res., **6**, 101 (1916).
JUCKERNACK (A.). *Volatile and Insoluble Fatty Acids in Butter.* Chem. Rev. Fett. Harz. Ind., **6**, 112–13 (1899).
KOEFOED. *Composition of Butterfat.* Analyst, **17**, 130 (1892).
MASSON (C.). *Variations in the Composition of Butter.* Bull. Assoc. Belge Chim., **12**, 108–9 (1898).
PAGNOUL (A.). *Observations on the Composition of Butters.* Bull. de l'Assoc. des Chim. de Suer et de Dist., **19**, 414–18 (1901).
SEBELIEN (J.). *The Composition of Old, Rancid Butterfat.* Landw. Vers. Sta., **79–80**, p. 389.
SIEGFELD (M.). *An Investigation on the Composition of Butterfat.* Zeits. Nahr. Genussm., **24**, 453–63 (1913).
SMEDLEY (I.). *The Fatty Acids of Butter.* Biochem. J., **6**, 451–61 (1913).
THOMPSON (S. C.). *Normal Composition of American Creamery Butter.* U.S. Dept. Agric. Bur. An. Ind., *Bull.* **149** (1912).
VUAFLART (L.). *Abnormal Butter.* Chem. Zentralb., 1918 (2), 299–300.
WANKLYN (J. A.). *On the Constitution of Butter.* J.S.C.I., **10**, 89–93 (1891).

INFLUENCE OF FEEDING ON BUTTER

BAUMERT (G.) and FALKE (F.). *Alterations in the Character of Butter by Feeding with Fat.* Zeits. Nahr. Genussm., **1**, 665 (1898).
ECKLES (C. H.) and PALMER (L. S.). *Influence of Underfeeding.* Missouri Agr. Expt. Statn. *Research Bull.* **25**, 107 pp. (1916).
—— ——. *Influence of Overfeeding. Loc. cit., Research Bull.* **24**, 34 pp. (1916).
—— —— and SWELT. *Factors Influencing the Composition of Milk.* Loc. cit., *Bull.* **151**, 33–5 (1917).
FORBES. *Mineral Metabolism of the Milch Cow.* Monthly Bull., Ohio Agric. Expt. Statn., **3**, 8–10 (1918).
MCCANDLISH. *Possibilities of Increasing Milk and Butterfat Production by the Administration of Drugs.* J. Dairy Sci., I., 475–86 (1918).
MOORE (F. W.). *Abnormal Butter.* J. Anal. and Appl. Chem., **7**, 1–2 (1893).
PUTNEY and LARSON. *Some Nitrogen Studies with Dairy Cows in Milk.* Ann. Rept., Penna State Coll., Agr. Expt. Statn., **1914–5**, 250–93 (1916).

RUFFIN (A.). *On the Influence of Feeding on Some of the Constants of Butter.* Ann. Chim. Anal., **4**, 383 (1899).
SJOLLEMA (B.) and TULLEKEN (J. E.). *Halphen's Reaction for Cottonseed Oil.* Zeits. Nahr. Genussm., **5**, 914 (1902).

SPECIFIC FOODSTUFFS

BOES and WEYLAND. *The Influence of Sugar-Beet Feeding.* Zeits. Nahr. Genussm., **29**, 473–5 (1915).
CRANFIELD (H. T.). *Influence of Feeding with Coconut Cake and Linseed Cake.* Analyst, **36**, 445–9 (1911).
——. *Decorticated Ground Nut Cake and Cottonseed Cake.* Analyst, **41**, 336–9 (1916).
—— and TAYLOR. *Linseed and Hempseed Cakes.* Analyst, **40**, 433–9 (1916).
—— ——. *Dried Yeast and Decorticated Cottonseed Meal.* Analyst, **41**, 240–5 (1916).
CROWTHER (J.). *Palm Kernel Cake.* J. Board Agric., **23**, 734–49 (1916).
ECKLES (C. H.) and PALMER (L. S.). *Cottonseed Oil Products.* Missouri Agr. Expt. Statn., *Research Bull.* **27**, 44 pp. (1916).
EWING and SPENCE. *Coconut Meal versus Cottonseed Meal for Dairy Cows.* Texas Agric. Expt. Statn., *Bull.* **225**, 1–9 (1918).
HOOPER (J. J.) and NUTTER (J. W.). *Feeding with Velvet Beans, Palm Kernel Meal, and Various Grain Mixtures.* Kentucky Agr. Expt. Statn., *circ.* **23**, 31–38 (1918).
RAMM (E.) and MÖLLER (E.). *Food for Milch Cows: Brewery Settlings.* Milch. Zeit., **28**, 97–99 (1899).
SIEGFELD (M.). *Influence of Coconut Cake and Beetroot Leaves.* Chem. Zeit., **31**, 511 (1907).
SHIELDS, RAITT and LIPSCOMB. *A Study to Determine the Cause of the Gummy Texture Produced in the South, Noting Especially the Influence of Various Rations on Texture and Flavour.* S. Carolina Station. Report for **1917**, pp. 1–16.
SMITH, WELLS and EWING. *Influence of Cottonseed Oil Feeding.* Georgia Agr. Expt. Statn., *Bull.* **122**, 95–111 (1916).
THORPE (T. E.). *Influence of Feeding with Cottonseed and Sesamé-seed Cakes.* Ann. Rept. for Distribution of Grants for Agric. Educ. (1897–8).
WAUTERS (J.). *The Detection of Cottonseed and Sesamé Oils in Fats.* Bull. de l'Assoc. belge Chim., **13**, 404 (1899).
WAYTS. *Soybean Meal and the Quality of Butter.* Engrais, **27**, 1166 (1913).

RENOVATED BUTTER

COCHRAN (C. B.). *Butter and Butter Adulterants.* J. Frank. Inst., **147**, 85–97 (1899).

CRAMPTON (C. A.). *The Brown-Taylor-Richards Method for the Microscopical Identification of Butter.* J. Amer. Chem. Soc., **22**, 703–5 (1900).

——. *Composition of Process or Renovated Butter.* J. Amer. Chem. Soc., **25**, 358–66 (1903).

HESS (W. H.) and DOOLITTLE (R. E.). *The Detection of Renovated Butter.* J. Amer. Chem. Soc., **22**, 150–2 (1900).

HUMMEL (J. A.). *An Examination of Brown and Taylor's Official Method of Identifying Butter.* J. Amer. Chem. Soc., **22**, 327–9 (1910).

POLLATSCHEK (P.). *Manufacture of Clarified Margarine.* Chem. Rev. Fett. Harz. Ind., **10**, 53–4 (1903).

SHAW and NORTON. *Renovated Butter.* J. Dairy Sci., I, 127–35 (1917).

TONNEY and STOCKTON. *The Effect of Renovation of Butter upon the Reichert-Meissl Number and the Saponification Value.* Amer. J. Public Health, **4**, 1083–85 (1914).

Patents—

ABEL. Eng. Pat. 9105 (1893).
ADAIR. Eng. Pat. 4495 (1888).
ARTUS. Eng. Pat. 20,027 (1893).
BOULT. Eng. Pat. 7500 (1907).
BREDENKAMP. Fr. Pat. 375,075 (1907).
CAMPBELL. Eng. Pat. 15,086 (1894).
CHRISTIANSEN. Eng. Pat. 8422 (1907).
CORDEWEENER. Eng. Pat. 24,461 (1893).
DUBUISSON. Eng. Pat. 28,374 (1904).
FAY. U.S. Pat. 847,562 (1907).
GREELEY. U.S. Pat. 784,394 (1905).
HARRIS. Eng. Pat. 9252 (1898).
HIGGINS. Eng. Pat. 15,221 (1913).
JENSEN. U.S. Pat. 881,929 (1908).
JULIEN. Eng. Pat. 23,637 (1898).
LAKE. Eng. Pat. 18,137 (1902).
MARCHAND. Eng. Pat. 4062 (1886).
MCDONNELL. Eng. Pat. 3233 (1873).
MONTÉRAN. Fr. Pat. 396,282 (1908).
MORGANS. Eng. Pat. 24,714 (1895).
RIEGEL. Eng. Pat. 12,095 (1907).
ROOS. U.S. Pat. 854,383 (1907).
TICHENOR. Eng. Pat. 2229 (1883).
WHITE. U.S. Pat. 797,481 (1905).

CHAPTER X

ANALYSIS OF BUTTER AND MARGARINE

I. Moisture-Content

Aschman (C.) and Arend (J. P.). *Direct Determination of Water in Butter and other Fats.* Chem. Zeit., **30**, 953 (1906).

Brauer (K.). *Moisture Contents of Margarines made from Hardened Oils.* Zeitsch. öffentl. Chem., **22**, 209–16 (1917).

Buttenberg (P.). *Proportion of Water in Margarine.* Zeitsch. Nahr. Genussm., **13**, 542–4 (1907).

Brünnich (J. C.) and Smith (F.). *The Determination of Moisture in Butter.* Queensland Agric. Jour., **28**, 9.

Frank (L.). *Rapid Determination of Water in Margarine.* Chem. Zeit., **43**, 314–15 (1919).

Hunziker (O. F.), Mills (H. C.) and Spitzer (G.). *Moisture Control of Butter.* Purdue Univ. Agric. Expt. Statn., *Bull.* **159** (16), 258–360; *Bull.* **160** (16), 365–419.

Koenig (F.). *Different Methods of Estimating Moisture in Butter.* Apoth. Zeit., **28**, 63 (1913).

Lindermann (O.). *Water Content of Salted Butter.* Milch. Zentr., **41**, 751–3 (1912).

Monhaupt (M.). *Determination of the Water Content of Margarine.* Chem. Zeit., **43**, 385–6 (1919).

Rivett (A. C. D.). *Estimation of Water in Butter.* Chem. News, **104**, 261 (1911).

Solstein (P.). *Determination of Fat, Non-Fatty Substances, and Water in Butter.* Chem. Rev. Fett. Harz. Ind., **12**, 125 (1905).

Vandebelde (A. J.) and Stewart (A.). *Water in Butter.* Bull. Soc. Chim. Belg., **23**, 95 (1909).

Wauters (J.). *Water in Butter.* Bull. Soc. Chim. Belg., **20**, 365–73 (1906).

II. Preservatives

Bonnet (F.). *Colorimetric Determination of Formaldehyde in Milk and Butter.* J. Amer. Chem. Soc., **27**, 601–5 (1905).

Cornalba (G.). *Boric Acid in Butter, and its Identification.* Bull. chim. farm., **51**, 433–5 (1913).

Friese (W.). *Estimation of Benzoic Acid in Margarine and Butter.* Pharm. Zentralhalle, **52**, 1201 (1911).

Halphen (G.). *Detection of Benzoic Acid in Butter.* J. Pharm. Chim., **29**, 201–3 (1908).

Hawley. *Boric Acid in Butter.* Analyst, **40**, 150 (1915).

Monhaupt (M.). *Detection and Determination of Boric Acid in Butter.* Chem. Zeit., **29**, 362–3 (1905).

PLANCHON and VUAFLART. *Detection of Borax in Butter.* J. Pharm. Chim., **4**, 49–51 (1896).
RICHMOND (H. D.) and HARRISON (J. B. P.). *Rapid Estimation of Boric Acid in Butter.* Analyst, **27**, 179 (1902).
—— and MILLER (E. H.). *Estimation of Boric Acid in Butter.* Analyst, **32**, 144 (1907).
RIMINI (E). *Detection of Formaldehyde in Foodstuffs.* Chem. Centralb. I. (1898), 1152.
ROBIN (L.). *Detection of Benzoic Acid in Butter.* Ann. Chim. Anal., **13**, 431–3 (1908).
VOLLHASE (E.). *Detection of Preservatives in Butter, Margarine and Lard.* Chem. Zeit., **37**, 312 (1913).

III. COLOURING MATTERS

AYRES (S. H.). *A Simple Butter Color Standard.* U.S. Dept. Agric., Bur. An. Ind., *circ.* **200** (1912).
BIANCHI. *Investigation of Foreign Colouring Matters in Butter.* Annali. Chim. Applic., **5**, 1–13 (1916).
BURGER. *Artificial Colour in Food Products.* Colour Trade J., **3**, 282–5 (1918).
CORNELISON (R. W.). *Detection of Synthetic Colours in Butter.* J. Amer. Chem. Soc., **30**, 478–81 (1908).
CRAMPTON (G. A.) and SIMONS (F. D.). *Detection of Palm Oil when used as a Colouring Matter in Fats and Oils.* J. Amer. Chem. Soc., **27**, 270 (1905).
DOOLITTLE (R. E.). *Distinction Between Annatto and Coal Tar Dyes.* U.S. Dept. Agric., Bur. of Chem., *Bull.* **65**, 152.
GEISLER (J. F.). *Delicate Test to Detect a Yellow Azo-dyestuff used for the Artificial Colouring of Edible Fats.* J. Amer. Chem. Soc., **20**, 110–113 (1898).
GILL (A. H.). *The Crampton-Simons' Colour Test for Palm Oil.* J. Ind. Eng. Chem., **9**, 136 (1917).
——. *Occurrence of Carotin in Oils and Vegetables.* J. Ind. Eng. Chem., **10**, 612–14 (1918).
HOUGHTON (H. W.). *Effect of Colouring Matters on Some of the Digestive Enzymes.* J. Amer. Chem. Soc., **29**, 1351–57 (1907).
KHLOPIN (G. V.) and BASIL'EVA (P. I.). *Method of Distinguishing between Natural and Artificial Coal-Tar Dyes.* J. Russ. Phys. Chem. Soc., **45**, 802–5 (1913).
LEEDS (F. H.). *Methods of Separation of Colouring Matters in Butter.* Analyst, **22**, 150 (1887).
LOOMIS (H. M.). *Identification of Food Colours.* U.S. Dept. Agric.; Bur. Chem., *circ.* **63**, 1–69 (1911).
LOW (A. H.). *Testing for an Azo-dye in Fats.* J. Amer. Chem. Soc., **20**, 889 (1898).
LUBS (H. A.). *Detection of Colour Added to Butter or Margarine.* J. Ind. Eng. Chem., **10**, 436–9 (1918).

MARTIN (E. W.). *Detection of Artificial Colouring Matters in Butter and Oleomargarine.* Analyst, **12**, 70 (1887).
MOORE (R. W.). *A Test for Carrot Colour in Butter.* Analyst, **xi**. 163 (1886).
PALMER (L. S.) and ECKLES (C. H.). *Carotin, the Principal Natural Yellow Pigment of Milk Fat.* J. Biol. Chem., **17**, 191–210.
—— and THRÜN (A.). *Detection of Natural and Artificial Pigments in Oleomargarine and Butter.* J. Ind. Eng. Chem., **8**, 614–18 (1916).
PRICE (T. M.). *Method for the Separation of the Seven Permitted Coal-Tar Colours in Mixtures.* U.S. Dept. Agric.; Bur. An. Ind., *circ.* **180**, July (1911).
RIDEAL (S.). *An Investigation of Certain Substances Used in Colouring Foods.* Lancet, June 10 and 17 (1911).
SCHMITT (E.). *Artificial Butter Colourings.* Ann. Agronomiques, **9**, 255–7 (1884).
VANDRICKEN (J.). *The Colouring Matter of Butter and Substitutes.* Ann. Pharm., **7**, 110 (1901).

IV. FOREIGN SUBSTANCES

CRAMPTON (C. A.). *Glucose in Butter.* J. Amer. Chem. Soc., **20**, 201–6 (1898).
FENDLER (G.). *Browning and Frothing of Margarine on Heating.* Chem. Rev. Fett. Harz. Ind., **xi**. 122–4 (1904).
GEISLER (J. F.). *Adulteration of Oleomargarin with Paraffin.* J. Amer. Chem. Soc., **21**, 605–8 (1899).
HERTKORN (J.). *Poisonous Substances in Vegetable Butters and Fats.* Chem. Zeit., **34**, 1381–2 (1910).
MECKE. *Detection of Yolk of Egg in Margarine.* Zeits. öffentl. Chem., **5**, 231 (1899).
——. *Detection and Estimation of Cane Sugar in Margarine.* Zeits. öffentl. Chem., **5**, 496 (1900).
REINSCH (A.). *Characteristics of Cardamon Oil and of the Fat of Margarine Prepared Therefrom.* Chem. Zeit., **35**, 77 (1911).

V. ANALYSIS OF BUTTER

A.—*Refractometric Methods*

AMAGAT (E. H.) and JEAN (F.). *Optical Analysis of Oils and Butters.* Comptes rendus, **109**, 616–17 (1890).
BEHREND (P.) and WOLFS (H.). *Relation between the Various Constants of Butterfat.* Zeits. Nahr. Genussm., **5**, 689–719 (1902).
BREEN (A. G.). *The Refraction of Butterfat and its Non-volatile Fatty Acids.* Zeits. Nahr. Genussm., **15**, 79 (1908).

DUMITRESCU (G.) and POPESCU (D. M.). *Refraction of the Insoluble Fatty Acids of Butter.* Ann. Falsific., **3**, 149–53 (1910).

ELLINGER (H. O. G.). *Optical Analysis of Butter and Margarines.* J. prakt. Chem., **44**, 157–9 (1891).

JEAN (F.). *Notes on the Optical and Chemical Analysis of Butter.* Monit. Scient. (1892), pp. 91–8.

——. *The Application of the Oleorefractometer to the Detection of Adulterations.* Bull. Soc. Chim., **4**, 105–8 (1890).

PARTHEIL (J.) and VELSEN (J. v.). *Refractometric Examination of Butter.* Arch. der Pharm., **238**, 261–79 (1900).

RICHMOND (H. D.). *Temperature Correction of the Zeiss Butyro-refractometer.* Analyst, **32**, 44 (1907).

SWAVING (A. J.). *Practical Use of the Refractometer in the Examination of Butter.* Chem. Centralb. (1898), I. 352.

THORPE (T. E.). *Interdependence of the Physical and Chemical Criteria in the Analysis of Butterfat.* J. Chem. Soc., **85**, 248–56 (1904).

VIOLLETTE (C.). *Optical Analysis of Butter.* Comptes rendus, **111**, 348 (1890).

B.—*Density Methods*

BRULLÉ (R.). *Method of Determining Purity of Butter by Means of the Density.* Comptes rendus, **122**, 325 (1896).

——. *A General Method for Testing Butter.* Comptes rendus, **116**, 1255 (1893).

BRUYN (C. A. L. DE). *Brullé's Method for Testing Butter.* Chem. Zeit., **18**, 1341–2 (1894).

PERKINS (A. E.). *Apparatus and Method for Determining the Hardness of Butterfat.* J. Ind. Eng. Chem., **6**, 136–41 (1914).

SELL. *Specific Gravity of Butter, Margarine, and Other Fats.* Milchw. Zeit., **15**, 460–1 (1887).

SOHN (C. E.). *Note on a New Method of Examining Butter.* Analyst, **18**, 218–21 (1893).

VIOLLETTE (C.). *Analysis of Commercial Butters.* Comptes rendus, **117**, 856–8.

WOLCKENAAR. *Determination of the Specific Gravity of Butter and Tallow at* 100° *C.* Rep. Anal. Chem., **5**, 236 (1885).

C.—*Viscosity Methods*

KILLING (C.). *Simple Method of Detecting Foreign Fats in Butter.* Chem. Zeit., **18**, 314 (1894).

POLENSKE. *Testing of Butter by Viscosity Measurements.* Apoth. Zeit., **xi.** 97 (1896).

WENDER (N.). *Viscosimetric Examination of Butter for Foreign Fats.* J. Amer. Chem. Soc., **17**, 719 (1895).

WHITE (G. F.) and TWINING (R. H.). *The Fluidity of Butterfat and its Substitutes.* J. Ind. Eng. Chem., **5**, 568–73 (1913).

D.—*Turbidity Methods*

BARTHOLOMÉ (J.). *Butter from the Physical Standpoint.* J. pharm. Soc. Anvers., **70**, 81–91 (1914).

CRISMER (L.). *Critical Temperature of Solution (Butter Analysis) in Open Tubes.* Bull. Assoc. Belge. d. Chim., **10**, 312–16 (1896).

ERDELYI (J.). *The Detection of Foreign Fats in Butter.* Zeits. anal. Chem., **31**, 407–10 (1892).

GRIMME (C.). *Critical Solution Temperature of Fats and Oils.* Seifensieder Zeit., **46**, 379–81 (1919).

HOUZEAU (A.). *Adulteration of Butter.* Comptes rendus, **116**, 952–6.

LÉZÉ (R.). *Detection of Margarines in Butter.* Comptes rendus, **112**, 813–15.

LOUISE (E.). *Applicability of the Miscibility Curves to the Analysis of Butter and Margarine.* Ann. Falsific., **4**, 302–5 (1911).

STEWART. *The Crismer Test for the Detection of Foreign Fats in Butter.* J. State Med. (U.S.A.), **26**, 312–15 (1918).

VANDAM (L.). *The Crismer Value of Butterfat.* Ann. Falsific., **12**, 260–9 (1919).

E.—*Cryoscopic Methods*

CARLINFANTI (E.) and LA FACE (F.). *Investigation of Butter.* Boll. chim. farm., **52**, 431–5 (1914).

PAILHERET (F.). *Cryoscopy of Fats, Especially Butter and Margarine.* Bull. Soc. Chim., **5**, 425–8 (1909).

QUARTAROLI (A.). *Distinction of Natural from Artificial Butter.* Staz. sperim. agrar. ital., **37**, 18–23 (1904).

F.—*Margarine Fats in Butter*

BELLIER (J.). *Butter Analysis.* Ann. Chim. anal. appl., **xi.** 412–24 (1906).

BONBZYNSKI (S.) and RUFI (H.). *Butterfat.* Zeits. anal. Chem., **29**, 1–6 (1890).

CASSALL (N. C.) and GERRAUS (B. H.). *Estimation of Coconut Oil in Admixture with Butterfat.* Analyst, **35**, 519 (1910).

COCHRAN (C. B.). *Detection of Foreign Fats in Butter and Lard.* J. Amer. Chem. Soc., **19**, 796–9 (1897).

DEGUIDE (C.). *Separation of Margarine from Butter.* J. Pharm. Chim., **16**, 372–7 (1902).

EWERS (E.). *Detection of Palm Kernel Oil, Coconut Oil, and Lard in Butter.* Zeit. öffentl. Chem., **16**, 131–41; 147–52 (1910).

FENDLER (G.). *Detection of Coconut Oil in Butter and Lard* Zeit. öffentl. Chem., **16**, 152–66 (1910).

FERREIRA (J.). *Apparently Sophisticated Butter.* Chem. Zeit., **36**, 589 (1912).

FLEISCHMANN (W.). *The Accuracy of Hehner's Method of Testing Butter.* Milch. Zeit., **16**, 731 (1887).

HANŮS (J.). *Detection of Coconut Oil in Butter.* Zeits. Nahr. Genussm., **13**, 18–24 (1907).

—— and STAKL (L.). *The Ethyl Ester Value: A New Constant for Detecting Coconut Oil.* Zeits. Nahr. Genussm., **15**, 577–87 (1908).

—— and PETRIK (E.). *Modified Determination of the Ethyl Ester Value in Butter Analysis.* Chem. Zeit., **34**, 736 (1910).

HARRIS (F. W.). *Determination of Coconut Oil in Butter.* Analyst, **31**, 353–60 (1906).

HEPNER (A.). *Detection of Coconut Oil in Butter by Fendler's Method.* Zeits. Nahr. Genussm., **21**, 754 (1911).

HESSE (H.). *Determination of Fat in Butter.* Zeits. Nahr. Genussm., **8**, 673–5 (1904).

HINKS (E.). *Detection of Coconut Oil in Butter.* Analyst, **32**, 160 (1907).

HODGSON (T. R.). *Detection of Coconut Oil in Butterfat.* Chem. News, **96**, 273, 288, 297 (1907).

INDEMANS (W. G.). *Coconut Oil in Butter and Margarine.* Bull. Assoc. Belge. d. Chim., **14**, 404–7.

JUCKENACK (A.) and PASTERNACK (R.). *Detection of Coconut Oil in Butter and Lard.* Zeits. Nahr. Genussm., **7**, 173–214 (1904).

KLENZE (v. W.). *Determination of Commercial Butterfat.* Z. landw. Vers. Wes. Öst., **4**, 77–80.

LEDENT. *Adulteration of Butter by Addition of Coconut Oil.* Bull. soc. chim. belge., **27**, 325–30 (1913).

LUDWIG (W.) and HAUPT (H.). *Detection of Coconut Oil in Butter.* Zeits. Nahr. Genussm., **13**, 605–10 (1907).

MCGILL (A.). *Butter.* Lab. Inland Rev. Dept., Ottawa, Can., *Bull.* **281** (1914).

MATTHÉS (H.) and ACKERMANN (E.). *Unsaponifiable Constituents of Coconut Oil, and Detection of that Fat in Butter.* Berichte, **41**, 2000–1 (1908).

MICKO (C.). *Estimation of Margarine in Butter.* Chem. Zeit., **17**, 113 (1893).

MILLER (E. H.). *The Aldehyde Figure of Butter.* Analyst, **37**, 50 (1912).

MONHAUPT (M.). *Determination of Butterfat in Presence of Coconut Oil.* Chem. Zeit., **33**, 305–6 (1909).

MÜNTZ (A.) and COUDON (H.). *Determination of Coconut Oil in Butter.* Monit. Scient., **18**, 530–41 (1904).

NILSON (L. F.). *Butter Analysis.* Zeit. anal. Chem., **28**, 175–83 (1889).

NOCKMANN (E.). *Detection of Coconut Oil in Butter by Ewer's Method.* Zeits. Nahr. Genussm., **21**, 758 (1911).

PAAL (C.) and AMBERGER (C.). *Metallic Salts of the Volatile Fatty Acids of Butterfat and Coconut Oil.* Zeits. Nahr. Genussm., **17**, 1–22 (1909).

PAAL (C.) and AMBERGER (C.). *Detection of Coconut Oil in Butter.* Loc. cit., **17**, 23–51 (1909).
ROBIN (L.). *Detection of Coconut Oil and Margarine in Butter.* Comptes rendus, **143**, 512–14 (1906).
——. *Detection of Adulteration in Butter.* Ann. Falsific., **5** 180–7 (1912).
ROSS (R.). *Estimation of Coconut Oil in Butter.* Analyst, **33**, 457 (1908).
—— and RACE (J.). *Estimation of Coconut Oil in Butter.* Chem. News, **97**, 110 (1908).
SCHEFFER. *Pure Butter and Artificial Butter.* Pharm. Rundsch., **4**, 248 (1887).
SEIDENBERG (A.). *Method of Detecting Foreign Fats in Butterfat.* J. Ind. Eng. Chem., **10**, 617–21 (1918).
VANDAM (L.). *Detection of Coconut Oil in Butter and Margarine.* J. Pharm. Chim., **6**, 273 (1901).
WAUTERS (J.). *Detection of Coconut Fat in Butter.* Bull. Soc. Chim. Belge., **19**, 6–7 (1905).
WIJSMANN (H. R.) and REIJST (J. J.). *Process for Detecting Coconut Oil in Butter.* Zeits. Nahr. Genussm., **xi.** 267–71 (1906).
WOLT (F. W. A.). *Butter Analysis.* Zeits. anal. Chem., **26**, 28–33 (1887).

VI. ANALYSIS OF MARGARINE

ARNOLD. *Oleomargarines and their Butterfat Content.* Zeits. Nahr. Genussm., **27**, 379–88 (1914).
ARNAUD (F. W. F.) and HAWLEY (H.). *Determination of Butterfat and Coconut Oil in Margarine.* Analyst, **37**, 122–3 (1912).
ASBÓTH (A. v.). *Discrimination between Butter and Margarine.* Chem. Zeit., **21**, 312–14 (1897).
ENVERS (N.). *Detection and Estimation of Arachis Oil.* Analyst, **37**, 487 (1912).
JOLLES (A.) and WILD (E.). *Sunflower Oil in Margarine.* Chem. Zeit., **17**, 879 (1893).
KÖNIG (L.) and BURBERG (W.). *Use and Determination of Butter Substitutes in Baker's Goods.* Zeits. Nahr. Genussm., **27**, 775–84 (1914).
LEYS (A.). *Determination and Identification of Small Quantities of Butterfat and Cocoafat in Edible Fats.* Ann. Chim. anal., **18**, 140–4 (1911).
REVIS (C.) and BOLTON (E. R.). *Methods of Detecting Coconut Oil in Butter and Margarine.* Analyst, **36**, 333–40 (1911).
RUSSELL (E.) and KIRKHAM (V. H.). *Characteristic Values of Margarine.* Analyst, **29**, 206–8 (1904).
SETTIMI (L.) and MAURANTONIS (L.). *Investigation of Vegetable Oils in Margarine.* Giorn. farm. chim., **61**, 452–3 (1913).

SEYDA AND WOY. *Comparison of the Analytical Methods for Detecting Margarine in Butter.* Chem. Zeit., **18**, 906 (1894).
UTT (C. A. A.). *Some Data on Peanut Margarine.* J. Ind. Eng. Chem., **6**, 746–7 (1914).
UTZ. *Distinction of Vegetable and Animal Fats.* Chem. Umschau. Fette, Oele, Wachse, Harze, **25**, 13–14 (1918).
VIRCHOW (C.). *Method of Distinguishing between Genuine and Artificial Butter.* Rep. Anal. Chem., **6**, 489 (1886).
VIOLLETTE (C.). *Analysis of Butter and Margarine.* Comptes rendus, **111**, 345–7 (1890).
VITOUX AND SALLE. *The Composition of Margarines.* Ann. falsific., **7**, 121–30 (1914).
WALLENSTEIN (F.). *On Margarines.* Chem. Zeit., **16**, 883–4 (1892).

VII. DISTILLATION OF FATTY ACIDS

ARNOLD (W.). *Precautions to be Taken in Determining the Polenské Value.* Zeits. Nahr. Genussm., **23**, 389–91 (1912).
BARTHEL and SONDEN. *Detection of Coconut Oil in Butter by Polenské Test.* Mon. sci., **8**, 268–72 (1918).
BEAL (W. H.). *The Determination of Volatile and Insoluble Fatty Acids in Butterfat.* J. Amer. Chem. Soc., **16**, 673–6 (1894).
BLICHFELDT (S. H.). *Proposed Method for the Estimation of Butter and Cocoafat in Margarine.* J. Soc. Chem. Ind., **29**, 792–4 (1910).
——. *Process for the Estimation of Butterfat, Coconut Fat, Palm Kernel Fat, and their Mixtures.* J. Soc. Chem. Ind., **38**, 150–152 (T.) [1919].
BOLTON (E. R.), RICHMOND (H. D.) and REVIS (C.). *Relation between the Kirschner and Polenské Values for Margarine Containing Coconut Oil or Palm Kernel Oil.* Analyst, **37**, 183–8 (1912).
BURNETT (H. R.) and REVIS (C.). *The Proportionate Determination of Coconut and Palm Kernel Oils in Mixture.* Analyst, **38**, 255 (1913).
BURR and WEISE. *The Free Fatty Acids and the Volatile Fatty Acids of Fresh Butterfat.* Molkerei Zeit., **28**, 291–2 (1914).
BÜNTE (C.). *Notes on some Modifications of the Reichert-Meissl Method of Detecting Margarine in Butter, and a New Method for the Determination of the Reichert-Meissl Value.* Chem. Zeit., **18**, 204–6 (1894).
CRANFIELD (H. T.). *On the Relation between the Reichert-Meissl, Kirschner, and Polenské Values for Butter.* Analyst, **40**, 439 (1915).
DELAITE (J.) and LEGRAND (J.). *Determination of Soluble and Insoluble Volatile Fatty Acids in Butter.* Bull. Soc. Chim. Belg., **20**, 230–5 (1906).

DONS (R. K.). *Caprylic Acid Values of Butterfat.* Zeits. Nahr. Genussm., **14**, 333–42 (1907).

——. *Determination of the Caprylic Acid Value of Butterfat.* Zeits. Nahr. Genussm., **15**, 75–9 (1908).

ELSDON (G. D.). *The Shrewsbury and Knapp Process for Estimating Coconut Oil.* Analyst, **42**, 72 (1917).

ERCULISSE and DACKWEILER. *New Method for the Analysis of Butter.* Ann. Chim. anal., **23**, 225–34 (1918).

FODOR (K. VON). *The Caproic Acid of Butterfat.* Zeits. Nahr. Genussm., **26**, 641–4 (1913).

HESSE (A.). *Polenské's New Butter Value.* Milchw. Zentr., I, 13–20 (1915).

JEAN (F.). *The Silver Value Method for the Detection of Coconut Fat in Butter.* Ann. Chim. anal. appl. **xi.** 121–4 (1906).

JØRGENSEN. *The Determination of Butterfat in Margarines.* Ann. falsific., **9**, 260–70 (1916).

KIRSCHNER (A.). *Determination of Butterfat and Coconut Oil in Margarine.* Zeits. Nahr. Genussm., **9**, 65 (1905).

KREISS (H.). *The Determination of the Reichert-Meissl Value of Fats by Means of Glycerol Potassium Hydroxide.* Chem. Zeit., **35**, 1053 (1911).

LEFFMANN (H.) and BEAM (W.). *A Modification of the Reichert Distillation Process.* Analyst, **20**, 153 (1891).

POLENSKÉ (E.). *Detection of Coconut Oil in Butter and Lard.* Arb. Kais. Gesundh., **38**, 402–14 (1911).

REICHER (L. T.). *Low Reichert-Meissl Value of Dutch Butter.* Zeits. angew. Chem., (1901), 125–9.

REILLY (J.) and HICKINBOTTOM (W. J.). *Determination of Volatile Fatty Acids by an Improved Distillation Method.* Sci. Proc. Roy. Dublin Soc., **15**, 513–38 (1919).

RICHMOND (H. D.). *Relation between the Kirschner, Reichert-Meissl, and Polenské Values of Butter.* Analyst, **44**, 166–7 (1919).

RIDEAL (S.) and HARRISON (H. G.). *The Polenské Method for the Detection of Coconut Oil in Butter.* Analyst, **31**, 254–8 (1906).

REYCHLER (A.). *Note on the Reichert Value of Butter and other Fats.* Bull. Soc. Chim., **25**, 142 (1901).

ROSS (R.) and MANDSLEY (F.). *Examination of Shrewsbury and Knapp's Process for the Estimation of Coconut Oil.* Analyst, **36**, 195 (1911).

SCHMIDT. *Analysis of Butter.* Chem. Zeit., **16**, 1275 (1892).

SHREWSBURY (H. S.) and KNAPP (A. W.). *A Short Method for Detecting and Estimating Coconut Oil in Butter and Margarine.* Analyst, **35**, 385–92 (1910).

——. *Shrewsbury and Knapp Process for Determining Coconut Oil.* Analyst, **37**, 3–7 (1912).

SUNDBERG (T.). *The Calculation of the Quantity of Coconut Oil Present in a Butter from the Polenské Value of the Mixture.* Zeits. Nahr. Genussm., **26**, 422 (1913).

TATTOCK (R. R.) and THOMSON (R. T.). *Value of the Polenské Test in the Analysis of Oils and Fats.* J. Soc. Chem. Ind., **28**, 690 (1909).

VUAFLART. *Analysis of Butters: Soluble Volatile Acids and Total Soluble Acids.* Ann. falsific., **7**, 367–9 (1914).

WITZEMANN (E. J.). *Volatility with Steam of Lower Fatty Acids in Dilute Aqueous Solution.* J. Amer. Chem. Soc., **41**, 1946–51 (1919).

WOLLNY (R.). *The Reichert-Meissl Method of Testing Butter, and its Applications for Controlling the Sale of Butter and its Substitutes.* Milch. Zeit. (1887), 32–5.

WRAMPELMEYER (E.). *Estimation of all the Volatile Fatty Acids in Butter.* Chem. Zentr., **2**, 986 (1897).

VIII. IODINE VALUE

DUBOVITZ (H.). *Preparation of the Wij Iodine Solution.* Chem. Zeit., **38**, 1111 (1914).

EPHRAIM (J.). *On the Hübl Method of Iodine Absorption.* Zeits. angew. Chem. (1895), p. 254.

HANŮS (J.). *The Use of Iodine Bromide in the Analysis of Oils and Fats.* Zeits. Nahr. Genussm., **4**, 913 (1901).

INGLE (H.). *Reactions Taking Place in the Determination of the Iodine Value.* J. Soc. Chem. Ind., **21**, 587 (1902).

MERGEN (W.) and WINOGRADOFF (A.). *The Action of Halogens on Oleic Acid, and Estimation of the Iodine Number of Fats.* Zeits. angew. Chem., **27**, 241 (1914).

PONZIO (G.) and GASTALDI (C.). *Relationship between the Iodine Value and the Structure of the Oleic Series of Acids.* Gaz. chim. ital., **42**, 92 (1912).

TOLMAN (L. M.) and MUNSON (L. S.). *Iodine Absorption of Oils and Fats.* J. Amer. Chem. Soc., **25**, 244 (1903).

UENO (S.). *Preparation of Wij's Iodine Solution.* J. Soc. Chem. Ind., **35**, 367 (1916).

WIJS (J. J.) *The Reactions in Hübl's Method of Iodine Absorption.* Zeits. angew. Chem., **xi.**, 291 (1898).

——. *A New Method of Determining the Iodine Absorption of Oils.* Berichte, **31**, 750 (1898).

——. *A Comparison of the Methods of Iodine Absorption in the Analysis of Fats.* Chem. Rev. Fett. Harz. Ind., **6**, 5 (1899).

——. *On the Determination of the Iodine Value.* Analyst, **25**, 31 (1900).

——. *The Use of Iodine Bromide in the Determination of the Iodine Value.* Zeits. Nahr. Genussm., **5**, 497 (1902).

WEISER (St.) and DONATH (H. C.). *Comparison of the Various Methods of Estimating the Iodine Value of Fats.* Zeits. Nahr. Genussm., **28**, 65 (1914).

CHAPTER XI

DETERIORATION OF BUTTER AND MARGARINE IN STORAGE

Rancidity

AMTHOR (C.). *The Causes of Butter Rancidity.* Zeits. anal. Chem., **38**, 10–20 (1899).

BROWN (C. A.). *Chemistry of Rancidity in Butterfat.* J. Amer. Chem. Soc., **21**, 975–99 (1899).

CRAMPTON (C. A.). *Influence of the Growth of Mold on the Chemical Composition of Oleomargarine.* J. Amer. Chem. Soc., **24**, 711–19 (1902).

GUTHRIE (E. S.). *Concerning Rancidity in Butter.* J. Dairy Sci., I., 218–34 (1917).

HANUS (J.). *Contributions to the Nature of Butter Acidity.* Zeits. Nahr. Genussm., **3**, 324–8 (1900).

—— and STOCKY (A.). *On the Chemical Action of the Growth of Molds in Butter.* Zeits. Nahr. Genussm., **3**, 606 (1900).

HUNZIKER and HOSMANN. *Tallowy Butter: its Causes and Prevention.* J. Dairy Sci., I., 320–46 (1917).

ISSOGLIO (G.). *The Analysis of Rancid Fats.* Ann. Chim. Appl., **6**, 1–18 (1916).

JENSEN (O.). *Rancidity of Butter.* Centr. Bakt. Parasitenk., **8** (2), 12 papers.

KERR. *Chemical Tests for the Detection of Rancidity.* J. Ind. Eng. Chem., **10**, 471–5 (1918).

KREGTEN (VAN). *Rancidity of Fats.* Chem. Weekblad., **15**, 992–3 (1918).

LIDOW (A.) and DSHORS. *Conversion of Butter into a Tallowy Product under the Influence of Light.* Chem. Zeit. (1903), **27**, Rep. 253.

MAYRHOFER (J.). *Rancidity of Butter and the Detection of Formaldehyde therein.* Zeits. Nahr. Genussm. (1898), pp. 552–3.

MJOEN (J. A.). *The Cause of the Rancidity of Fats.* Forsch. Ber., **4**, 195–203 (1897).

NAGEL (J.). *Separation of the Constituents of Rancid Fats and Oils.* Amer. Chem. J., **23**, 173–6 (1900).

REINMANN (R.). *Rancidity of Butter.* Centr. Bakt. Parasiteuk., **6** [2], 166, 209.

ROGERS (L. A.) and GRAY (C. E.). *Influence of Cream Acidity on Butter Flavour.* U.S. Dept. Agric., Bur. An. Ind., *Bull.* **114**.

SALKOWSKI (E.). *Rancidity of Fat.* Zeits. Nahr. Genussm., **34**, 305 (1919).

SCALA (A.). *Rancidity in Fats.* Gaz. chim. ital., **38** [1], 307–27 (1908).
SCHMID (A.). *Testing Fats for Rancidity.* Zeits. anal. Chem., **37**, 301–3 (1898).
SLYKE (L. L.) and HART (E. B.). *The Proteids in Cream, Butter, and Buttermilk in Relation to Mottled Butter.* J. Amer. Chem. Soc., **27**, 679–90 (1905).
SMITH (T.). *Rancidity of Fats.* Pharm. J., **95**, 4–5 (1915).
SÖHNGEN (L. N.). *Influence of Fat-splitting Microbes on Margarine.* Beitr. z. gesamt. Mikrobiol., I., 199–248 (1913).
SOLTSIEN (P.). *The Chemical Detection of Rancidity in Butter.* Chem. Rev. Fett. Harz. Ind., **12**, 177 (1905).
SPAETH (E.). *On the Rancidity of Fats.* Zeits. anal. Chem., **35**, 471 (1896).
WEIGMANN and WOLFF. *New Observations on the Origin of the Turnip Taste in Butter.* Landw. Jahrb., **46**, 343–65 (1914).
WHITE (H. L.). *The Modification of the Composition of Vegetable Oils, with Special Reference to Increasing Unsaturation.* J. Ind. Eng. Chem., **xi.**, 648–51 (1919).
WIEDMANN (F.). *Detection of Rancid Fat in Edible Fats.* Zeits. Nahr. Genussm., **8**, 136 (1904).
ZOFFMANN (A.). *Micro-organisms in Margarine.* Chem. Rev. Fett. Harz. Ind., **xi.**, 7 (1904).

STORAGE CHANGES

BLANCHET. *Enzyme Activity at Cold-Storage Temperature.* Le Froid, **5**, 6–9 (1917).
BROWN. *Decomposition of Casein in Presence of Salt by Butter Flora.* Science, **42**, 319 (1915).
—— and PEISER. *Keeping Qualities of Butter.* Mich. Agric. Expt. Statn., *Tech. Bulls.* **29**, 5–19 (1916); **30**, 5–18 (1916).
CRISPO (D.). *Supposed Loss of Soluble Volatile Acids during the Storage of Butter.* Bull. Soc. Chim. Belg., **24**, 436–8 (1910).
COMBS AND ECKLES. *Relation of Oidium lactis and Penicillium chrysogenius to the Keeping Qualities of Butter.* J. Dairy Sci., I., 347–55 (1917).
DYER. *Progressive Oxidation of Cold-Storage Butter.* J. Agric. Res., **6**, 927–52 (1916).
HUNZIKER. *The Pasteurization of Sour Farm-skimmed Cream for Butter-making.* Purdue Univ. Agric. Expt. Statn., *Bull.* **20**, No. 208 (1917), 1–76. (Discusses cold-storage effects in butter.)
——. *Protein Hydrolysis in Fresh and Stored Butter.* Indiana Stat., Report for 1917, pp. 35–6.
KILDEE (H. H.). *Cold Storage of Butter.* Minnesota Agr. Expt. Statn. Report, **25**, 48–9 (1917).

LAXA (O.). *Decomposition of Butterfat by Micro-organisms.* Arch. Hyg., **41**, 119–151.

NESTRELAJEW (A.). *Changes in Butterfat under the Influence of Light.* Milch. Zentralb., **6**, 1–8 (1910).

RUATA (G. Q.). *Action of Cold on Micro-organisms.* Bull. Agric. Intell., **10**, 116 (1919).

SAGER, RAHN, and FERRAND. *Keeping Properties of Butter in Cold Storage.* Centr. Bakt. Parasiteuk., 1908 [ii.], 22.

THATCHER and DAHLBERG. *The Influence of Salt on the Changes Taking Place in Storage Butter.* J. Dairy Sci., I., 114–26 (1917).

ROGERS (L. A.). *Keeping Qualities of Canned Butter.* U.S. Dept. Agric. Bur. An. Ind., *Bull.* **57**.

——. *Factors Influencing the Change in Flavour of Storage Butter.* U.S. Dept. Agric. Bur. An. Ind., *Bull.* **162**, 5–69.

USE OF PRESERVATIVES

BORDAS (F.). *Preservation of Butter by Means of Sodium Bicarbonate and Sodium Nitrate.* Ann. falsif., **7**, 45–9 (1914).

FETTICK. *Preserving Action of Salt.* Centr. Bakt. Parasitenk., 1908 [ii.], 22, 32.

FISCHER (K.) and GRUENERT (O.). *Influence of Certain Preservatives on the Keeping Qualities of Butter and Margarine.* Zeits. Nahr. Genussm., **22**, 553–82 (1911).

GRIMALDI (C.). *The Influence of Preservatives on the Reichert-Meissl Value of Fats.* Annuli del. Lab. Chem., **6**, 631 (1912).

IRIBARNÉGARAY (B.). *Preservation of Butter with Sodium Fluoride.* Eng. Pat. 3830 of 1897.

KIPPENBERGER (C.). *Is the Addition of Preservatives, Notably Benzoic Acid, Permissible in Margarine?* Zeits. angew. Chem., **26**, 66–8 (1913).

RUMI. *Influences on Health of Antiseptics Added to Foods.* Anales soc. quim. Argentina, **6**, 210–14 (1918).

TANNER (F. W.) and FUNK (R. S.). *Some Observations on the Use of Boric Acid as a Disinfectant.* Amer. J. Pharm., **91**, 206–10 (1919).

TREVE (E.). *A Starch Syrup containing Sulphite in Margarine.* Zeit. Nahr. Genussm., **24**, 742 (1913).

VALLÉE (C.). *Case of Poisoning by Sodium Fluoride.* J. Pharm. Chim., **21**, 5–8 (1920).

WEBER (H. A.). *Behaviour of Antiseptics towards Salivary Digestion.* J. Amer. Chem. Soc., **14**, 4–14 (1892).

WEIGLE (T.) and MERKEL (S.). *Action of Formalin on Foodstuffs.* Chem. Zeit. (1895), p. 142.

OFFICIAL REPORTS. *Report of the Dept. Comm. Appointed to Inquire into the Use of Preservatives and Colouring Matters in the Preserving and Colouring of Food.* [Parliamentary Blue Book. Eyre and Spottiswoode, 1901.]

OFFICIAL REPORTS. *Effect of Boric Acid on Milk.* Govt. Bur. of Microbiology, N.S.W. Rept. (1909), pp. 124–130.
——. *Dyes, Chemicals, and Preservatives in Foods.* U.S. Dept. Agric., Food Inspection Division, **76** (1907).

CHAPTER XIII

NUTRITIONAL CHEMISTRY

General Papers

ABDERHALDEN (E.) and SCHAUMANN (H.). *Organic Foodstuffs with Specific Action.* Pflüg. Arch., **172**, 1–274 (1918).
BIERRY and PORTIER (P.). *Vitamines and Symbiotes.* Comptes rendus, **166**, 193–6 (1918).
BUHRER (C.). *Vitamines.* Schweiz. Apoth. Zeit., **57**, 377–80 (1919).
CHICK, HULME, and SKELTON. *Antiscorbutic Value of Cow Milk.* Biochem. J., **12**, 131–53 (1918).
CORNALBA. *Vitamines and New Ideas Regarding Human Nutrition.* Boll. chim. farm., **56**, 577–80 (1917).
DRUMMOND (J. C.). *Some Aspects of Infant Feeding.* Lancet (1918), **2**, 482–4.
——. *Rôle of the Antiscorbutic Factor in Nutrition.* Biochem. J., **13**, 77–80 (1919).
——. *Fat-Soluble Vitamine: its Nature and Properties.* Biochem. J., **13**, 81–94 (1919).
——. *Rôle of the Fat-Soluble Vitamine in Nutrition, and its Influence on Fat Metabolism.* Biochem. J., **13**, 95–102 (1919).
EDITORIAL. *Vitamine Hypothesis in Relation to Alleged Deficiency Diseases.* J. Amer. Med. Assoc., **68**, 6040–1 (1917).
EIJKMAN. *Importance of Vitamines in Human Nutrition.* Chem. Weekblad., **15**, 757–83 (1918).
FUNK. *Physiological Importance of Some Previously Unknown Food Constituents, the Vitamines.* Ergebnisse Physiol., **13**, 125–205 (1913).
GREEN. *Experimental Expression of the Relationship between the Content of a Foodstuff in Antineuritic Hormone, and the Period of Healthy Survival of Animals Upon It.* S. African J. Sci., **14**, 483–4 (1918).
HAMPSHIRE (C. H.) and HAWKER (G. E. G.). *Note on Vitamines.* Pharm. Jour., **103**, 82 (1919).
KREISSL. *Vitamines and their Relation to Nutrition.* Wien. Med. Wochsch. (1918), pp. 547–51.
MCCOLLUM (E. V.). *The Newer Knowledge of Nutrition.* [Macmillan, N. York, 1919.]

McCollum (E. V.). *Vitamine Hypothesis and Diseases Referable to Faulty Diet.* J. Amer. Med. Assoc., **71**, 937–40 (1918).

Mendel (L. B.). *New Points of View Regarding the Part Played by Different Food Substances in Nutrition.* J. Amer. Med. Assoc., **63**, 819–22 (1914).

Osborne (T. B.) and Mendel (L. B.). *Milk as a Source of Water-Soluble Vitamine.* J. Biol. Chem., **34**, 537–51 (1918).

——. *The Vitamines in Green Foods.* J. Biol. Chem., **37**, 187–200 (1919).

Ramsden (W.). *Vitamines.* J. Soc. Chem. Ind., **37**, 53–5, T. (1918).

Ranc (A.). *Bio-ernergenesis, and the Symbiotes Theory of Paul Portier.* l'Industrie chimique, **6**, 136–9 (1919).

Röhmann (F.). *Ueber Künstliche Ernährung und Vitamine.* (Berlin, 1916.)

Steenbock (I. H.). *Vitamines and Nutrition.* Sci. Monthly, **7**, 179–88 (1918).

——, Boutwell (P. W.) and Kent (H. E.). *Fat Soluble Vitamine.* J. Biol. Chem., **35**, 517–26 (1918).

—— and Boutwell. *Thermostability of Fat-soluble A in Plant Materials.* J. Biol. Chem., **41**, 163–171 (1920).

Stepp. *Indispensability of Lipoids for Life.* Z. Biol., **66**, 365–86 (1916).

Voegtlin. *Importance of Vitamines in Relation to Nutrition in Health and Disease.* J. Wash. Acad., **6**, 579–95 (1916).

Weill (E.) and Mouriquand (G.). *Dietary Deficiency Diseases* J. Amer. Med. Assoc., **62**, 1510–1 (1914).

Vitamines in Butter and Margarine

Funk and Macallum. *The Comparative Value of Lard and Butter in Growth.* J. Biol. Chem. **27**, 51–62 (1916).

Halliburton (W. D.) and Drummond (J. C.). *Nutritive Value of Margarines and Butter Substitutes with Reference to their Content of the Fat-Soluble Accessory Growth Substance.* J. Physiol., **51**, 235–51 (1917).

McCollum (E. V.), Simmonds (N.) and Pitz (W.). *Distribution in Plants of Fat-Soluble A, the Dietary Essential of Butterfat.* Amer. J. Physiol., **41**, 361–75 (1916).

—— and Davis (M.). *Isolation of the Substance in Butterfat which Exerts a Stimulating Influence on Growth.* J. Biol. Chem., **19**, 245–50 (1914).

——, Simmonds (N.), and Steenbock (I. H.). *A Method for the Separation of the Dietary Essential, Fat Soluble A, from Butterfat.* J. Biol. Chem., **29**, 26 (1917).

—— and Simmonds (N.). *A Study of the Dietary Essential, Water-Soluble B, in Relation to its Solubility and Stability towards Reagents.* J. Biol. Chem., **33**, 55–89 (1918).

OSBORNE (T. B.) and MENDEL (L. B.). *The Stability of the Growth-Promoting Substance in Butterfat.* J. Biol. Chem., **24**, 37–9 (1916).

RUITER (J. DE). *Lipoids and Vitamines in Margarines and Butter.* Nature, **96**, 704 (1916). J. Ind. Eng. Chem., **8**, 1020 (1916).

DIGESTIBILITY OF FATS

ANON. *Edible Cottonseed Oil.* Chem. and Druggist (1899), p. 370.

BOUROT and JEAN. *Comparative Digestibility of Cocoanut Butter and Cow's Butter.* Comptes rendus, **123**, 587–90 (1896).

GARDNER (J. A.) and FOX (F. W.). *Digestibility of Cocoa Butter.* Biochem. J., **13**, 368–77 (1919).

HOLMES (A. D.). *Digestibility of Some Seed Oils.* U.S. Dept. Agric., *Bull* **687**, 20 pp. (1918).

——. *Studies on the Digestibility of Some Nut Oils.* U.S. Dept. Agric., *Bull.* **630**, 19 pp. (1918).

——. *Digestibility of Certain Miscellaneous Animal Fats.* U.S. Dept. Agric., *Dept. Bull.* **612** (1919).

JOLLES (A.). *Margarine Compared with Natural Butter as Regards Digestibility and Nutritive Value.* Monatsch. Chem., **16**, 147–63 (1894).

LANGWORTHY and HOLMES. *Digestibility of Some Animal Fats.* U.S. Dept. Agric., *Bull.* **310** (1915).

MAIGNON (F.). *Study of the Mechanism of the Action of Fat in the Utilization and Assimilation of Proteins.* Comptes rendus, **168**, 474–6 (1918).

OVERMAN (O. R.). *Food Values and Dairy Products.* Ill. Agric. Expt. Stat., *circ.* **235**, 1–28 (1919).

ROCKWOOD and SWICKES. *Relative Digestibility of Maize Oil, Cottonseed Oil, and Lard.* J. Amer. Med. Assoc., **71**, 1649–50 (1918).

See also *Edible Hydrogenated Oils*, p. 150.

OFFICIAL IDENTIFICATION OF MARGARINE

HART (F.). *Margarine Rendered Conspicuous by Adding Phenolphthalein.* Chem. Zeit., **17**, 1908–9 (1893).

HENRIQUES (R.). *The Question of Colouring Margarine.* Chem. Rev. Fett. Harz. Ind., **4**, 68–70 (1897).

MAINSBRECQ. *Denaturing Margarine.* Bull. Assoc. Belge des Chim., **12**, 185–7 (1898).

NEUFELD (C. A.). *Detection of Sesamé Oil in Butter and Margarine in the Presence of Artificial Colouring Matters.* Zeits. Nahr. Genussm., **3**, 156–8 (1893).

PARTHEIL (A.). *Addition to Margarine of Dimethylamido-azobenzene.* Chem. Zeit., **21**, 255–6 (1897).

RAUMER (VON). *Sesamé Oil cannot be used for the Recognition of Margarine.* Zeit. angew. Chem (1897), pp. 749–51.

SIEGFELD (M.). *Sesamé Oil in Margarine.* Chem. Zeit., **22**, 319–21 (1898).

SOLSTEIN (P.). *Detection of Sesamé Oil in Butter and Margarine.* Pharm. Zeit., **42**, 837 (1897).

WAUTERS (J.). *Denaturing Margarine.* Bull. Assoc. Belge des Chim., **12**, 58–69 (1898).

LEGAL

WHITE (J.). *Legal Limits for Butter.* J. Dairy Sci., I., 378–9 (1918).

WOLLNY (R.). *The Reichert-Meissl Method of Testing Butter, and its Applications for Controlling the Sale of Butter and Margarines.* Milch. Zeit. (1887), pp. 32–5.

BUTTER AND MARGARINE ACT, 7 Ed. VII. c. 21, *vide* Analyst, **32**, 399 (1907).

OFFICIAL REPORT. *The Margarine Clause of the Food and Drugs Act.*, vide Analyst, **25**, 309 (1900).

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